A REPORT ON A CYTOLOGICAL INVESTIGATION OF SOME BEES AND WASPS (HYMENOPTERA) WITH SPECIAL REFERENCE TO THEIR SEX DETERMINING MECHANISM

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I. INTRODUCTION

The present paper is a report on an investigation into the nuclear cytology of certain bees and wasps. It brings to light evidence for the existence of a differential chromosome - evidence which appears to be sufficiently strong to warrant a re-orientation of our views of the sex determining mechanism in the Hymenoptera. These views are now compatible with those held in regard to other organisms.

The paper itself contains four main parts. First, there is a historical review which provides the background to the study and establishes the existence of certain phenomena typical of the Hymenoptera. The most important of these are parthenogenesis, an atypical spermatogenesis, and the existence of germ-line and somatic polyploidy. The second section contains the record of the cytological investigations of bees and wasps. The third section is a general summary and is followed by the fourth, a discussion.

Material for the work has been obtained from two sources. The vespoid wasps and bumble bees are those found in the district and it is a pleasure to thank Mr. L.J. Lehr, of Crewe, for prompt communication of the sites of their nests and for many other acts of kindness. Queen honey bees, on the other hand, came from Sicily. They were remarkable for their docility and prolificness and my deepest gratitude goes to M.A. Alber for granting me the privilege and delight of using them.

In the course of the years during which this investigation has been proceeding I have constantly enjoyed the encouragement of Prof. F.E. Zeuner to whom I wish to express my most sincere gratitude. My special thanks, too, go to Prof. R.J. Pumphrey F.R.S. for granting me the privilege of working in his Department, and to Mrs. R. C. Bisbee, of this Department, for her critical interest in the work and for her assistance to me in every possible way. To Mr. R.A. Flemming and to Mr. W. Irving I would tender my thanks for struggling with those photographs which were beyond my power to produce, and to Mr. P.J. Alden and Mr. C. Grange I likewise express my sincerest appreciation of their ready co-operation with all problems of equipment.

II. HISTORICAL REVIEW

For convenience, this review is presented in four parts. There is first a general survey designed to focus the attention on the problem of parthenogenesis in the Hymenoptera; the second part is a cytological survey covering the fields of chromosome number and behaviour; the third deals with the more recent investigations relevant to sex determination; the last is a discussion of polyploidy. All four aspects naturally lend themselves to separate treatments, though all inevitably contribute to the picture as a whole.

1. Parthenogenesis, its occurrence in bees and wasps.

The elucidation of many of the early problems of the Hymenoptera was achieved through the use of the honey bee (Apis mellifera L.) as the experimental type. Parthenogenesis is a case in point, although the actual phenomenon itself was first discovered by Bonnet in Aphids in the year 1740. The realisation that it also existed in the honey bee came a little over 100 years later as a result of the investigations of Dzierzon who, from 1845 until 1876, maintained, what was afterwards proved, that a drone is the product of an unfertilized egg, the workers and queens (females) being derived from fertilized eggs. Naturally much pioneer work preceded this discovery. Thus towards the last quarter of the 17th century (circa 1670) the microscopist Jan Swammerdam established the sex of bees, and in the latter part of the 18th century (circa 1789), at a

time when Shirach was experimenting with queen rearing, the blind naturalist François Huber gave us our first scientific introduction to the mating problems of the honey bee. Fraser (1951), however, rightly maintains that Anton Janscha, beekeeper to Maria Theresa, anticipated by some 18 years (circa 1771) many of the discoveries attributed to Huber. From Huber's time another half century had still to elapse before the coming of Dzierzon. Moreover, while the latter appreciated the nature of parthenogenesis, he did not actually coin the term which was first used by von Siebold (1857) for the development of eggs without fertilization.

Following Dzierzon's discovery there came a period of considerable controversy involving the theories of Landois, of Kipping, of Pflüger, and, particularly in 1898 and the early 20th century, of Dickel (vide Nachtsheim, 1913). Dickel very vigorously opposed Dzierzon's theory, arguing that sex determination was solely the result of specialised secretions of worker bees penetrating newly-laid eggs, and that fertilization was uniform for all eggs. Nevertheless, supporters of Dzierzon were fairly numerous and among the earliest was von Siebold. He actually paid Dzierzon a visit in 1851 and, being much influenced by his conversation, he undertook an anatomical investigation into certain structures of the queen bee. His results appeared to him to provide adequate proof of the correctness of the Dzierzon theory. In 1857, therefore, he strongly maintained that in eggs destined to become workers sperms were present, whereas in those which would develop into drones, they were absent, Seven

years later von Siebold (1864) also believed that his observations on Eugster's gynandromorphs fell into line with, rather than contradicted, the Dzierzon theory and this point of view was later supported by Boveri (1888, and again in 1915) and Mehling (1915). Boveri, indeed, emphasised that the male parts of the gynandromorphs carried only maternal characters which could be recognised as such because the gynandromorphs themselves were race hybrids. On the other hand the female parts carried both maternal and paternal characters. He thus considered it likely that a male arose directly from an egg nucleus whereas a female would originate from a fusion nucleus.

Other supporters of Dzierzon included Leuckart and Berlepsch, and to a lesser extent such men as Gerstäcker, Bessels and Kulagin (vide Nachtsheim, 1913) and, in particular, Weismann and his pupils Paulcke (1899, 1900) and Petrunkewitsch (1901). In fact the latter's work brought the story of the honey bee considerably further forward and certainly broadened the scope of future investigations, which henceforth were mainly cytological or mainly genetical. Berlepsch (1851 and onwards) had really begun the latter type of investigation, or rather discussion, at a period when Dzierzon had temporarily lost faith in his own theory, and it was continued by Lowe (1867), Pérez (1878), Arviset (1878), Sanson (1878), Girard (1878), Matter (1879), Cook (1879) and finally summarised first by Taschenberg (1892) and later by Phillips (1903), Dalla Torre (1910), and Buttel-Reepen (1915).

The main reason for a distinction, at this stage, between genetical and cytological studies is that a real bone of contention

had arisen. Thus cytological evidence, that unfertilized eggs give males and fertilized ones females, seemed not to be supported by genetical evidence because the male did not appear to carry exclusively maternal characters. The genetical investigations, however, were based solely on body colour which was not considered by all ~~workers~~ investigators of this time to be a reliable enough guide. Indeed, colour alone is no guarantee of purity of race and, therefore, in spite of Pérez' (1889) contention to the contrary, it is quite possible that, unwittingly, earlier results were invalidated by the use of data based on the progeny of hybrid females. More recent genetical work by Cuénot (1909), Newell (1914), and Michailoff (1931) has still been based on body colour but the results have been used to support the cytological evidence. The best known is that of Newell who made reciprocal crosses between yellow Italian and grey Carniolan bees, obtaining results as follows (in Crew, 1933) :-

	Female		Male		Female	Male
	yellow	x	grey	=	yellow	yellow
	grey	x	yellow	=	yellow	grey
F_1	yellow	x	yellow	=	yellow :	50% yellow : 50% grey

Inheritance in the males was here purely matriclinous as would be expected if they arose from unfertilized eggs. The validity of the experiment, however, obviously derives from Newell's good fortune in possessing bees that apparently bred true for colour. It is, nevertheless, very easy to see how earlier investigators may

well have mistaken a hybrid, similar to that of the F_1 generation, for a pure yellow bee. Indeed, the conditions of experimental bee mating at this time could not prevent the drifting of drones, with the consequent lack of control over the characters of the offspring. To overcome this difficulty Michailoff (1931) employed artificial insemination for his crosses. He used the Watson technique (see below) but his results, judged by the number of progeny, indicate only a moderate degree of success with this technique. Nevertheless they certainly show most interesting genetic segregations. He also demonstrated a clear case of matriclinous inheritance - this was with his no.63 queen which, however, had mated naturally. This queen, whose mother was known to produce white-eyed drones, was herself shown, by means of a genetic analysis of her progeny, to be heterozygous for white-eye. Moreover, she must have received sperm from a black-eyed drone since her female progeny was shown to be either black-eyed heterozygotes or black-eyed homozygotes. Half her male progeny was black-eyed and half white-eyed, the latter evidently having been derived solely from herself.

The technique of artificial (instrumental) insemination has been perfected only in very recent years (for summary, see Laidlaw, 1949). Nevertheless, it was attempted as long ago as 1886 and, for genetic studies, in 1923 with the so-called Quinn-Laidlaw system. In 1927, however, L.R. Watson demonstrated the first effective method on queen bees; this was followed later by genetical experiments (Michailoff, 1931; Nolan, 1937) based on this method. The technique, however, never led to high degrees of success until

O. Mackensen and W.C. Roberts (1944) greatly improved it. They still based the technique upon the Watson method, but modified it on account of the re-discovery by Laidlaw (1944) of a previously overlooked obstructing valve (c.f. Bishop, 1920) in the genitalia of the queen. By means of this improved technique the Dzierzon theory, henceforth referred to as male haploidy or arrhenotoky, has been indisputably proved, but the actual experiments are incidental to other work and are scattered throughout beekeeping literature. A case in point is to be found in the experimental work of Rothenbuhler et alii (1952) who investigated gynandromorphs. They used queens, homozygous for the recessive ivory-eye gene, artificially inseminated with semen from drones hemizygous for the recessive gene for chartreuse eye. (Ivory and chartreuse were established as being non-allelic and non-linked). Female progeny from the matings were black-eyed (wild type) but the male progeny were ivory-eyed.

So far the discussion has centred on the honey bee. However, the beginning of the 20th century saw some attention paid to wasps (Meves, 1903; Mark and Copeland, 1907; Meves and Duesberg, 1908), to the bumble bee (Meves, 1903), to Xylocopa (Granata, 1909 and 1913), and to Osmia (Armbruster, 1913). The presence of male haploidy was strongly indicated in the entire group. Moreover, today, through extended studies of other groups the phenomenon is suspected throughout the whole of the Hymenoptera.

One further point remains to be discussed in this section. During investigations of the honey bee it was suspected by several

authors, though proof was difficult, that unfertilized eggs whether laid by a fecundated or by an unfecundated queen or by a laying worker, could give rise to a small proportion of female progeny (vide Anderson, 1918). The existence of such thelytokous females is now completely confirmed as a result of the work of Mackensen (1943). As early as 1912 Onions recognised that laying workers of Cape honey bees could produce females and his observations were confirmed by Jacks (1916). They sought a solution of the difficulty in a correlation of the size of the spermatheca with the suspected ability of the worker to be inseminated, though such insemination, in actual fact, was never proved and no spermatheca was ever seen to contain sperms. The present suggested explanation, discussed more fully later, is that a parthenogamy has taken place through fusion of the first polar nucleus with the egg nucleus.

From this general survey it is clear that biological experiment has established parthenogenesis in bees and wasps. Work outside the scope of this paper also indicates its presence in the Hymenoptera in general. It is almost completely arrhenotokous (confined to the male) but occasionally thelytoky makes its appearance with the production of females from unfertilized eggs.

2. Previous cytological investigations of bees and wasps.

Cytological investigations of wasps have covered only spermatogenesis but three vespoid wasps early claimed attention: 1) Vespula (= Vespa) germanica (Fab.) (vide Meves, 1903), 2) Vespula (= Vespa) maculata (Linn.) (vide Mark and Copeland, 1907), 3) Vespa crabro Linn. (vide Meves and Duesberg, 1908). The investigations are important for the light they shed on the character of the atypical spermatocyte maturation which, more than anything else, led to the realisation of the presence of male haploidy in the group. In wasps the first 'division' was found to consist of an omission of metaphase followed by the formation of an anucleated protoplasmic bud. The second maturation division, however, was completely equational leading to the production of two functional spermatids. During this stage, too, a supplementary spindle was observed but its explanation was not attempted. Chromosome numbers were never actually established though they were known to be not less than 16. On these main points there was essential agreement amongst the different workers although some slight differences of opinion were apparent between Meves and Duesberg on the one hand and Mark and Copeland on the other in regard to minor details.

The remaining investigation of wasps came much later and was concerned with four unnamed species of sphecoid wasps. It was undertaken by Whiting (1947b) and was extremely brief. Whiting merely reported two facts: 1) the existence in these species of but one meiotic division which produced two equal spermatids, and 2) the development, close to the nucleus, at the end of the growth period

before the meiotic division takes place, of a body resembling a second nucleus of small size. This body was reported as being very conspicuous, as persisting throughout the meiotic division, and as coming to occupy a position within the cytoplasm at one side of the cell. Whiting suggested that these phenomena be interpreted "as vestiges of the first division" but this statement contradicts, by implication, the reported existence of "but one meiotic division". In the present paper an exactly similar nucleus is described in vespoid wasps and has been found to be associated with the sex-determining mechanism.

Much more is known about the bees, since the number of investigators has considerably increased. Moreover, the range of observation is greater and includes oogenesis as well as spermatogenesis. It thus becomes necessary in this section to indicate the main problems that have arisen and the varying degrees of success in their solution that have so far been achieved. Oogenesis will be introduced first, since historically it has first claim to consideration.

As already mentioned, the first worker to study the honey bee cytologically, with modern techniques, was Petrunkewitsch (1901 - 1903). However, in 1889, Blochmann had discovered that not only the fertilized but also the unfertilized eggs produce both polar nuclei, the oocyte nucleus undergoing two maturation divisions with complete reduction. Ten years later Paulcke made a similar investigation, extended in 1900 - 1901 to include cell-differentiation in the ovary, and confirmed Blochmann's findings in a general way, the main

disagreement being over the origin of the polar nuclei. Blochmann maintained (in Phillips, 1903) that it was the second polar nucleus that divided, while Paulcke contended that it was the first. Such a controversy, however, has now a mere historical interest and is reviewed in Nachtsheim (1913). It is to Petrunkewitsch (op. cit.), who completed the observations begun by Paulcke, that we must turn for our first insight into chromosome behaviour in female cells and it is essential for us to consider his works carefully. Briefly stated they contain the following points:-

(a) the first enumeration of chromosomes, 16, from an oocyte metaphase plate and a statement that both drone and worker egg contain this same number:

(b) the belief that 8 chromosomes is the haploid number, and that at second maturation of the oocyte the chromosomes were reduced to this number with the final production of four chromosome groups. This was also believed to occur at spermatocyte maturation, which he did not investigate. To remove the various difficulties of this theory he considered that the unfertilized egg had a restoration of chromosome numbers by longitudinal splitting:

(c) the statement that in blastoderm cells of both sexes the chromosome number was increased to 64:

(d) the strange theory that the 'Richtungskopulationskern' or polar copulation nucleus - supposedly the fused product of the second polar nucleus and the median half of the divided first polar nucleus - gave rise in the male to germ cells. The latter, as the product of the fusion of a reduced first polar and second polar nucleus, would be diploid.

Oogenesis in the honey bee was further investigated, though at first very briefly, by Doncaster (1906) and, at about the same time, also by Hewitt (1906). Doncaster gave the chromosome numbers from the ovaries of young queens as about 16. Later Nachtsheim (1912) made a more complete investigation. The latter's best known paper, however, appeared in 1913. It is necessary to refer to this paper but before doing so the story of spermatogenesis must be brought up to date.

Meves (1903, and later 1907) was the first to investigate this problem, but about the same time Doncaster (1906 and 1907b), and Mark and Copeland (1906) also made similar investigations. There is considerable agreement in all accounts, but that of Meves (1907) is the fullest. Meves maintained that first maturation is abortive, the nuclear membrane remaining intact and a non-nucleated protoplasmic bud being formed. This bud, however, finally disappears and nothing comes of the cell activity. Second maturation is accompanied by a normal splitting and separation of the chromosomes but the division of the protoplasm is unequal, one nucleus remaining in the cell, the other passing into a protoplasmic bud. However, this bud after beginning to develop, is later sloughed off into the tubule to degenerate, only the large cell becoming a functional spermatid. Meves (1903) states that the same applies to the bumble bee, and it is now also known to be true for other bees such as Osmia (Armbruster, 1913) and Xylocopa (Granata, 1909 and 1913). Lastly, the chromosome number 16 (found later also in Osmia and Xylocopa) is not reduced during maturation. Meves

considered that the extrusion of the first, degenerate, non-nucleated bud from the germ cell of the honey bee is for the purpose (using a teleological argument) of maintaining the 16 chromosomes, a point already stressed by Giglio-Tos (1905). The latter, indeed, indicated that since the males are produced from unfertilized eggs containing only 16 chromosomes, any numerical reduction in the male germ track would render the germ cells incapable of fertilizing the eggs. Another important aspect of chromosome studies stressed at this time was the supposed tendency of chromosomes to couple or, in some cases, to from false tetrads. Doncaster (1906) especially mentioned this problem and at first maintained that 8 pairs existed, each consisting of two members of equal size which separated at second maturation. Later (1907b), however, he came to agree with Meves that the metaphase of the second maturation division exhibited 16 double chromosomes. Mark and Copeland (1906) differed from Meves, not on chromosome numbers, but in believing that the formation of the first non-nucleated bud was for the extrusion of the inter-zonal body. This body is a peculiar scar-like structure on the outside of the cell, and is now thought to be a relic of the tissue that once united spermatogonia in the cysts. Mark and Copeland, however, considered it to be the remnant of a former division spindle, and, therefore, doubted that the first spermatocyte 'division' was a maturation process. Since these authors were apt to be rather sparing in some of their observations of cell activities, particularly of the finer details of protoplasmic bud production, this aspect of their work must be treated with considerable caution.

We now come to Nachtsheim, and without question few investigators have exerted so profound an influence upon our attitude to the cytological problems of the honey bee as this author. To review his publications here in detail, however, is not warranted and, indeed, his obsevations of spermatogenesis do not differ fundamentally from those of Meves. Only the more important aspects of his investigation of oogenesis, fertilization and chromosome number need be considered.

According to him, egg maturation consists of two consecutive divisions accompanied by chromosome reduction. The spindle is already formed in the egg by the time it is laid and each chromosome group at anaphase contains eight dyads, the outermost group being the first polar nucleus. (Nachtsheim always considers a chromosome as a single body, but when they are paired or intimately associated in twos he speaks of a dyad). At second maturation, which immediately follows the first, the first polar nucleus also divides, with the result that four chromosome groups occur, the innermost being the pronucleus of the egg. The two middle groups are said to fuse to form the polar copulation nucleus which, after several divisions, degenerates, together with the dividing outer polar nuclei, and disappears. The pronucleus is now said to travel more deeply into the ooplasm and, in a fertilized egg, encounters a male pronucleus, with which it fuses. In the egg which has not been fertilized, the female pronucleus is said to continue its course transversely until it reaches the opposite side when it begins to divide and give rise to cleavage nuclei.

With regard to chromosome numbers, Nachtsheim maintained that the normal female complement is 32, which is reduced during maturation to 16. The latter, he claims, are 8 'double' chromosomes and not 16 bodies as such. The male germ cells also contain 16 chromosomes at all stages, there being no reduction of the number at maturation although secondary association was shown to occur. Unfortunately, Nachtsheim introduced the theory of 'Doppelchromosomen' whereby single and double values were given to chromosomes. He did this in order to bring his anomalous observations into a workable basic framework. Sanderson (1932) more fully discusses this aspect of Nachtsheim's work.

Nachtsheim (op. cit.) also maintained that in the honey bee from 3 - 7 sperms normally enter each egg, and he made it quite clear that he was referring to the entire spermatozoon, both head and tail being visible in the ooplasm (his figs. 12, 13, 14). He claimed that one spermatozoon penetrated deeply, and that a re-shaping of its head to a spiral capable of screw movement takes place. Following this the tail is soon lost and then the head begins to assume a dumb-bell shape, later rounding up into a ball from which plasma radiations extend into the ooplasm. He describes the supernumerary spermatozoa as attempting mitotic divisions on anastral spindles and sending out erratic radiations, but he believes that thereafter they soon perish.

The next important article came from the pen of Jegen (1920). He contributed arguments which are scarcely upheld today, although one aspect of his theory has a most interesting historical signific-

ance. He described a 'chromatoid' body, sometimes seen in duplicate, passing into one of the two spermatids, both supposedly functional: in this can be recognised an observation of what may be an extruded X-chromosome. Jegen, of course, was not alone in observing this body. Indeed, Nachtsheim (op. cit.) makes distinct reference to it and Armbruster (op. cit.) notes it several times in Osmia, even venturing to suggest that the body might be accredited with sex determining influence. In the wasps, too, its recognition came early (Meves and Duesberg, op. cit.). All the investigators were naturally very uneasy about its character but an analysis of their statements shows that it was rather the position of the body in relation to the main chromosome mass, and not the body itself, that caused the difficulty of interpretation. Indeed, Armbruster only dismissed the possibility that it was a heterochromosome because of its position 'outside' the spindle.

From 1921 until 1948, when Kerr (1948), Sanderson and Hall (1948 and 1951), and Manning (1949 and 1952a) re-opened the investigation, no further original work on the cytology of bees and wasps is known. This dearth, however, does not imply indifference to the study but is rather indicative of a change of outlook: related fields of study began to be explored (e.g. the study of sawflies, braconids, cynipids etc.); there was a tremendous widening of all cytological knowledge and techniques; and lastly quite a new approach developed, that of instrumental insemination, bringing the advantage of undisputed genetical fact to supplement cytological observations.

The paper by Kerr (op. cit.) was mostly concerned with the determination of caste but it nevertheless provided interesting cytological data relevant to the Meliponini. It included an account of spermatogenesis in Trigona and Melipona in which the main features are seen to be typical of the Apidae: it also reported the haploid number of chromosomes as 9 for this group.

The papers of Sanderson and Hall, on the other hand, gave a brief account of spermatogenesis and oogenesis in the honey bee. The 1948 paper maintained, first of all, that the chromosomes are well defined in shape and individuality, and that they are rod-shaped and not rounded as depicted by Meves, Doncaster, and Nachtsheim. They state that on 'gonial' (spermatogonial?) plates one chromosome is hooked and somewhat larger than the rest, and that in late anaphase it is very striking, They mentioned that hooked chromosomes, such as these, were described by Granata in Xylocopa violacea L., where all 16 are the same. With regard to the abortive division of the spermatocyte they merely confirmed that the chromosome number was not reduced during maturation. However, they failed to confirm the widely accepted views on bud formation. This was no doubt due to their unfortunate choice of the smear technique for the investigation.

They also state categorically "we have never found the chromosomes lying in pairs in either male or female and we therefore suggest that the apparent pairing of chromosomes and formation of 'Sammelchromosomen' found by Nachtsheim were due to the technique employed." In my own investigation, however, secondary association

of chromosomes has been clearly demonstrated, as will be seen below, and it is almost certain that it was their own unfortunate choice of the smear technique which prevented Sanderson and Hall from making similar observations.

Chromosome numbers were recorded by them in the spermatocyte and the oocyte as 16 and 32 respectively and, in addition, oogonial counts from queen larvae and pupae, and from worker larvae before the ovaries degenerate, were also reputed to be 32.

Sanderson and Hall's 1951 paper was in essence a re-affirmation of the contents of their 1948 paper.

The observations of Manning (op. cit.) on the honey bee, on the whole tended to support Nachtsheim's results after making due allowance for the earlier techniques employed by him. The results, however, were differently interpreted. Thus the association of spermatocyte chromosomes was attributed, first, to the presence of dyads and, secondly, to the effects of a past polyploidy. The 'coupling' of oocyte chromosomes ('doppelchromosomen') was interpreted as the close association of prophase chromosomes resulting from three phenomena - a previously unsuspected continued polarisation of these chromosomes, residual polyploidy, and the omission of chiasmata. The chromosome number in the female, however, was reported as 31 (= 30A/X; a differential oocyte maturation retaining the X-chromosome for the egg pronucleus). The 'chromatoid' body, which was also considered to be the 'hooked' chromosome of the spermatocyte recorded by Sanderson and

Hall, was interpreted as an X-chromosome extruded during spermatogenesis. This earlier work has been continued and greatly extended, and the results form the subject matter of the present paper.

From this survey of the cytological literature it is evident that, in spite of much contradiction on many points, certain facts emerge: 1) Oogenesis in both bees and wasps is, in the main, normal. 2) Spermatogenesis takes place in haploid germ cells in both bees and wasps: the first maturation is abortive and usually results in the formation of a non-nucleated protoplasmic elongation or bud: the second spermatocyte maturation division is equational in character, protoplasmically it is equal in wasps giving two functional spermatids but in bees it is unequal giving one functional spermatid and a small but nucleated bud which degenerates. 3) The presence of a 'chromatoid' body within the cytoplasm during maturation of the spermatocyte is recorded by several early workers. 4) Paired chromosomes and dyads have been observed.

3. Investigations relevant to sex determination in the Hymenoptera.

As we have already seen, early genetic experiment had, as its primary concern, the establishment of the presence of male haploidy, and cannot be said to have contributed in any direct way to the solution of the problem of sex determination, as such. The early cytological studies were also undertaken without any such object in mind. Indeed, it is doubtful whether the need to explain further the problem of sex determination ever occurred, since those investigators who had revealed the presence of the male haploid nucleus sought the explanation of sex in the haploid-diploid condition of male and female cells respectively. It was only after the work of Bridges on sex in Drosophila, in which a system of genic balance was found to be operative, that the 'ploidy' of the cell was found to be inadmissible in this context: haplo-diploidy could no longer be accepted as the explanation of sex in the Hymenoptera but only as a description of the state of the male and female genomes.

In fact, the only species in this Order for which we have a clear and precise account of sex determination is the braconid, Bracon hebetor Say (= Habrobracon juglandis (Ashm.)✗, described by P.W. Whiting and his collaborators. Between 1918 and 1943 they issued, at irregular intervals, solutions to the many subsidiary problems of their study, until finally they succeeded in establishing

✗Footnote. Since the name Habrobracon has become firmly established in cytological and genetical literature it is retained in the present paper.

an answer to the main problem. This came with the discovery of the operation of a series of sex alleles (9 were found). Females were heterozygous (xa/xb, xa/xc, xb/xc etc.): males, developing from the unfertilized eggs, were azygous (xa, xb or xc etc.). Fertilized eggs, of course, would be heterozygous or homozygous according to the cross, and Whiting found that, whereas the heterozygotes gave females, the homozygotes were mostly inviable and those that did survive gave diploid males (xa/xa, or xb/xb etc.). Inbreeding, inevitably involving a 2-allele cross, would thus give a high incidence of inviability amongst fertilized eggs: from xa/xb X xa almost half the fertilized eggs would be inviable. Such a mechanism, therefore, would be inapplicable to inbreeding species. Even Whiting himself admitted the unlikelihood of the general applicability of the Habrobracon scheme throughout the Hymenoptera, particularly after he had studied (1947a) the chalcid Melittobia chalybii Ashm.. Schmieder (1938) had previously investigated various aspects of the bionomics of this insect and, in his experiments, had consistently practised mother-son and sibling matings. Presumably because of this, Whiting later collaborated with Schmieder (1947) in testing the reproductive economy of the species. He found neither diploid males nor evidence of low viability in the inbred lines, and recognised that sex determination could not be on the Habrobracon system.

The case of the Scelionid Telenomus fariai Lima is also instructive since this too is incompatible with the system reported

for Habrobracon. Dreyfus and Breuer (1944) claimed that sex determination was the result of the distribution of X and Y chromosomes. They described a differential spermatogonial mitosis during which a segment was detached from the X-chromosome (they imply that it is extruded), leaving a residual segment which they call the Y chromosome. This Y was then retained for the sperm pronucleus. In addition a differential oocyte maturation was believed by them to retain the whole X-chromosome for the egg pronucleus. Dreyfus and Breuer also quote an earlier work of Dozorceva (1936a, b) where X- and Y- chromosomes were reported in the chalcid, Pteromalus puparum L.. As discussed later, the facts recorded by Dreyfus and Breuer appear to be quite compatible with a sex-determining mechanism of the type described in the present study for the honey bee.

Mackensen (1951 and 1952), however, attempted to extend the Habrobracon system to the honey bee. Using the instrumental insemination technique he made individual sibling matings. On the strength of high viability in the offspring he assumed that the original parental cross was three-allele (i.e. xa/xb X xc). Mating the daughters from the cross with their brothers he expected to obtain the following fraternities if the system were the same as that of Habrobracon :- In the first generation one half the fraternities should show high viability, the other half low : in the second generation, when breeding from the 'low viability' fraternity, low viability should again appear, but when breeding from the

'high viability' fraternity, results similar to those of the high viability first generation should be obtained. His results encouraged him to conclude that the sex-determining mechanism in the honey bee is the same as that of Habrobracon. It is nevertheless a fact that under natural conditions of inbreeding the high incidence of inviability in the honey bee, associated with the Habrobracon system, has never previously been reported in beekeeping literature even by bee-breeders with isolated breeding stations. On the contrary, the establishment of such breeding stations has been encouraged inorder to maintain purity of stock. Again, Mackensen claims that outbreeding is the rule but this is by no means established; indeed it is more usual to regard a queen as having the better chance of mating with a drone from her own hive. Lastly, the presence of biparental males, which are so necessary to prove relation of inviability to sex, has never been demonstrated and it thus seems rather premature to suggest that the presence of lethals in the honey bee are in any way associated with sex.

An entirely different view of the sex-determining mechanism in the honey bee, put forward by Manning (1949), has already been referred to above (p.21). According to this view sex is determined by a genic balance, X + 30 autosomes giving a female, X + 15 autosomes giving a male, the X being absent from the sperm as a result of a differential spermatogenesis but always present in the egg as a result of a differential oogenesis. Mackensen criticises this view on the strength of some recent facts brought to light by Rothenbuhler and his co-workers (1951 and 1952) in their work on

gynandromorphs. These authors showed, among other things, that, in gynandromorphs derived from an ivory-eyed queen inseminated with sperm from a chartreuse-eyed male, the male facets were chartreuse whereas the female facets were black. This appears to bring undisputed evidence that the origin of the male tissue of the gynandromorph is ~~from~~ from the sperm. Mackensen argues that supernumerary sperm pronuclei in the ooplasm, whose subsequent divisions should produce the sex mosaic, are, on Manning's hypothesis, without an X-chromosome and therefore female if they can develop at all. This line of reasoning, of course, is correct. Mackensen, however, failed to take cognisance of an alternative explanation. Sex mosaics are rare and thus it is desirable to indicate some exceptional circumstance under which the supernumerary sperm nucleus might remain viable in the ooplasm and divide to give the male elements of a sex mosaic. It seems most likely that the occasional ability of the supernumerary sperms to develop in this manner may be due to the fact that they indeed carry the X-chromosome as a result of an untoward failure to extrude this body from the nucleus at spermatogenesis. Such sperms would give male tissue and, in Rothenbuhler's cross, would also carry chartreuse eye. If, however, such exceptional sperms do occur it might also be expected that they would sometimes fuse with the egg nucleus, the result being, on the genic balance theory, a biparental male. Such males have not been recorded, but they would not bear any obvious mark of their double origin except from particular crosses, and even here, unless they were being specially looked for

they might well be missed. But it is also quite probable that, in competition with normal sperms, the abnormal X-carrying ones might never fuse with an egg nucleus: it is even just as probable that if they did, the resulting zygote might be inviable. In any case, it is difficult to see how anything in Rothenbuhler's results invalidates the idea of genic balance as the sex-determining mechanism in the honey bee.

It is evident from the present survey that two theories of sex determination in the honey bee remain in the field: that of 'haplo-viable homozygous lethals', operating as described by Whiting for Habrobracon, has been proposed by Mackensen; on the other hand Manning postulates a system of genic balance. The strength of Mackensen's theory derives from the discovery of the expected inviability in crosses of daughters with their brothers. The basis of Manning's theory lies in cytological observations which are further detailed in the present paper.

4. Polyploidy in the Hymenoptera.

It is to be expected that early investigators were unaware of the existence of polyploid tissues, and assumed that the number of chromosomes was the same throughout the organism. It was, therefore, natural that the discovery by Petrunkewitsch of 64 chromosomes in certain female and male cells, and the later discovery of anomalous numbers by Meves, Doncaster and Nachtsheim, should have been related at first to the problem of 'Sammel-chromosomen'.

Subsequent investigations, however, of the honey bee and of other members of the Hymenoptera have revealed that germ cells exhibit constant normal numbers in haplo-diploid ratio for male and female, the same condition probably prevailing also in normal connective tissue, muscle, nerve, intestinal tissue, and some blood cells. On the other hand, various degrees of polyploidy are exhibited by tracheal, fat, malpighial, coenocytic and hypodermal tissues, by some follicle cells, sheath cells of testes and ovaries, and by cells of developing wing buds, but whether in haplo-diploid ratio has not been reliably established. The most probable explanation of polysomy in the Hymenoptera is that endomitosis regularly occurs involving reduplication of chromosomes without cell division.

Of the two types of germ-line polyploidy postulated by different workers (also c.f. Whiting, 1945), one has never been observed, at least in the Hymenoptera. This is inconstant polyploidy whereby haploid and diploid numbers are first increased to higher

polyploid numbers and later reduced. Straight germ-line polyploidy, on the other hand, seems to be widespread in this group, the original monoploid numbers having been increased to polyploid in the course of evolution. Such derived polyploids may still show, cytologically, considerable trace of the polyploid condition (Greenshields, 1936a, b). This state of affairs is suspected in honey bees (Manning, 1952a), where a diplo-tetraploid, or even a tetra-octoploid complement of chromosomes may in actual fact exist. For practical purposes, however, it is usual to disregard the residual element of polyploidy and to consider the organism simply as haplo-diploid. This term merely implies that the germ-line nuclei of the male contain half the number of chromosomes seen in the germ-line of the female and does not reflect the actual state of the germ-line polyploidy.

Apparently, not only are the tissues of the honey bee and other members of the Hymenoptera typically haplo-diploid for male and female respectively but also there exists a polysomy and a straight germ-line polyploidy. There has been a tendency, particularly among the early authors, to confuse these forms.

III. PRESENT INVESTIGATIONS

1. Methods and techniques.

Methods of procedure adopted in the following investigations varied with the nature of the material and these have been more appropriately described under each section.

The general techniques employed consisted of squashes, sections and, only to a small extent, smears. Acetic orcein squashes were invariably found to give excellent results. For sectioned material the main fixatives used were acetic/alcohol (1:3), Carnoy, Flemming and Navashin, and the chief stain used was Haidenhain's iron haematoxylin. The latter was especially useful after acetic/alcohol. Crystal violet was used after non-alcoholic fixatives but only for demonstrating the presence of centrosomes. Feulgen was employed in order to obtain an insight into the deoxyribose nucleic acid content of the differential chromosomes but results were not entirely satisfactory. To discover whether the fault lay in the technique or in the material, check slides were made using marrow cells (human) obtained after sternal puncture. These gave clear configurations against poor results obtained from honey bee material. It was concluded that the unavoidable drop in temperature necessarily entailed when removing pupae from the hive may have been a contributary cause of the poorness of the results.

The duration of time that the material was left in the fixatives varied little from standard practice except in the case of acetic/alcohol and Carnoy. Even with these, normal practice was the rule; sometimes, however, when special development of spindle fibres was

desirable, long fixation was undertaken, extending over two or three weeks.

The microscope used was a Watson 'Bactil'. At first a Leitz 2mm. oil immersion objective was employed but this was later replaced by a Watson 2mm. fluorite oil immersion objective used with a holoscopic X10 eyepiece and holoscopic oil immersion condenser.

Drawings were made, where possible, using the camera lucida; where this was unsuitable the relative proportions and positions were first ascertained by photography, and then the details were filled in by direct observations. In the case of wasps, cells showing the supplementary spindle were selected for their uniformity and thus for their ease of comparison. This was thought desirable in view of the considerable range in size and strength this structure exhibits.

2. The Cytology of the Honey Bee and the Bumble Bee (Apidae).

(a) The Honey Bee, Apis mellifera L.

The present section contains an account of an extended investigation into the cytological problems of the honey bee, and includes the story of spermatogenesis and oogenesis together with a short discussion of chromosome number and behaviour, some reference to the behaviour of spermatozoa, and a preliminary discussion of sex determination.

Spermatogenesis.

One of the great advantages of the study of the honey bee is the ease with which, at the appropriate season, male germ track cells can be obtained at stages suitable for their cytological investigation. The method employed for obtaining drone brood as early in the year as possible was to introduce a frame of drone comb into the middle of the brood nest during a warm day in April and then to remove larvae and pupae when, and as, required. No special procedure is necessary from mid-May onwards since drone brood naturally becomes abundant as the season advances. Spermatocytes can be obtained from pupae in which colour is just appearing in the eye. Such pupae are from 11 - 13 days old and their cells have been completely capped over. Spermatogonia are readily obtained from very early pupae, and maturing spermatozoa from the older pupae. Spermatogenesis is almost complete when the pupal head is well-coloured and bundles of spermatozoa then lie, with tails free, in the tubules.

Testes are fortunately large enough to be easily dissected out, and in the present investigation they were either made into acetic orcein squashes, or placed in the various cytological fixatives mentioned above. The chief stains used were Haidenhain's iron haematoxylin, crystal violet and Feulgen. Of these three, iron haematoxylin, after either long or short fixation in acetic/alcohol, gave the most useful results.

Spermatogonia are easily recognised in their cysts. They are oval or pear-shaped and exhibit 16 chromosomes on their metaphase

plates. The last spermatogonial cell and the nucleus within it are both relatively large, the latter most often occupying a central position but sometimes lying asymmetrically. Nucleoli are not always easy to recognise after acetic orcein but are readily perceived after iron haematoxylin: at least two are known to exist. The last spermatogonial interphase is well characterised by a distinct nuclear membrane. Only as the metaphase gives place to the prophase of the primary spermatocyte, however, can the chromosome threads be recognised, prochromosomes having previously been very weak if present at all.

The prophase chromosomes carry distinct chromomeres (text-fig.1, no.1) and their chromatid split occurs early, rapidly followed by the separation of the parts. Nevertheless, the latter associate together as dyads and continue to do so from this time forward, with various degrees of closeness of the association, until their final movement apart, from the metaphase plate, at second maturation division.

Late prophase, metaphase and anaphase of the first maturation 'division' are anomalous. No true plate is formed although a very weak spindle can sometimes be observed. Moreover, the cell, at one pole, develops a distinct protoplasmic elongation which, however, is not terminated by a bud (Plate 1, nos.1,2; text-fig.1, nos.2,3). This protoplasmic elongation is fairly precise in its occurrence, consistent in its appearance, and well developed structurally. Its development is at first accompanied by a unipolar elongation of the

Explanation of text-fig. 1.

Maturation of the primary spermatocyte.

1. Early prophase showing large heteropycnotic chromosome; chromomeres present on the more centrally placed chromosomes; from acetic orcein preparation.

2. Primary spermatocyte showing the abortive metaphase plate, the scatter of chromosomes along the axis of the cell, and the intense staining cone of the protoplasmic elongation. The dyad structures are illustrated and the X-chromosome at 2 o'clock. From iron haematoxylin preparation.

3. Early interkinesis; nuclear membrane elongated; from acetic orcein preparation.

Maturation of the secondary spermatocyte.

4. Metaphase, side view, showing the regular plate of autosomes, and the detached position of the X-chromosome with its own spindle fibres; from preparation of iron haematoxylin, long fixation acetic/alcohol.

5. Anaphase, side view; X-chromosome on periphery of nuclear area, its own nuclear membrane beginning to form; main spindle S-shaped, more strongly formed at one pole; from preparation of iron haematoxylin, long fixation acetic/alcohol.

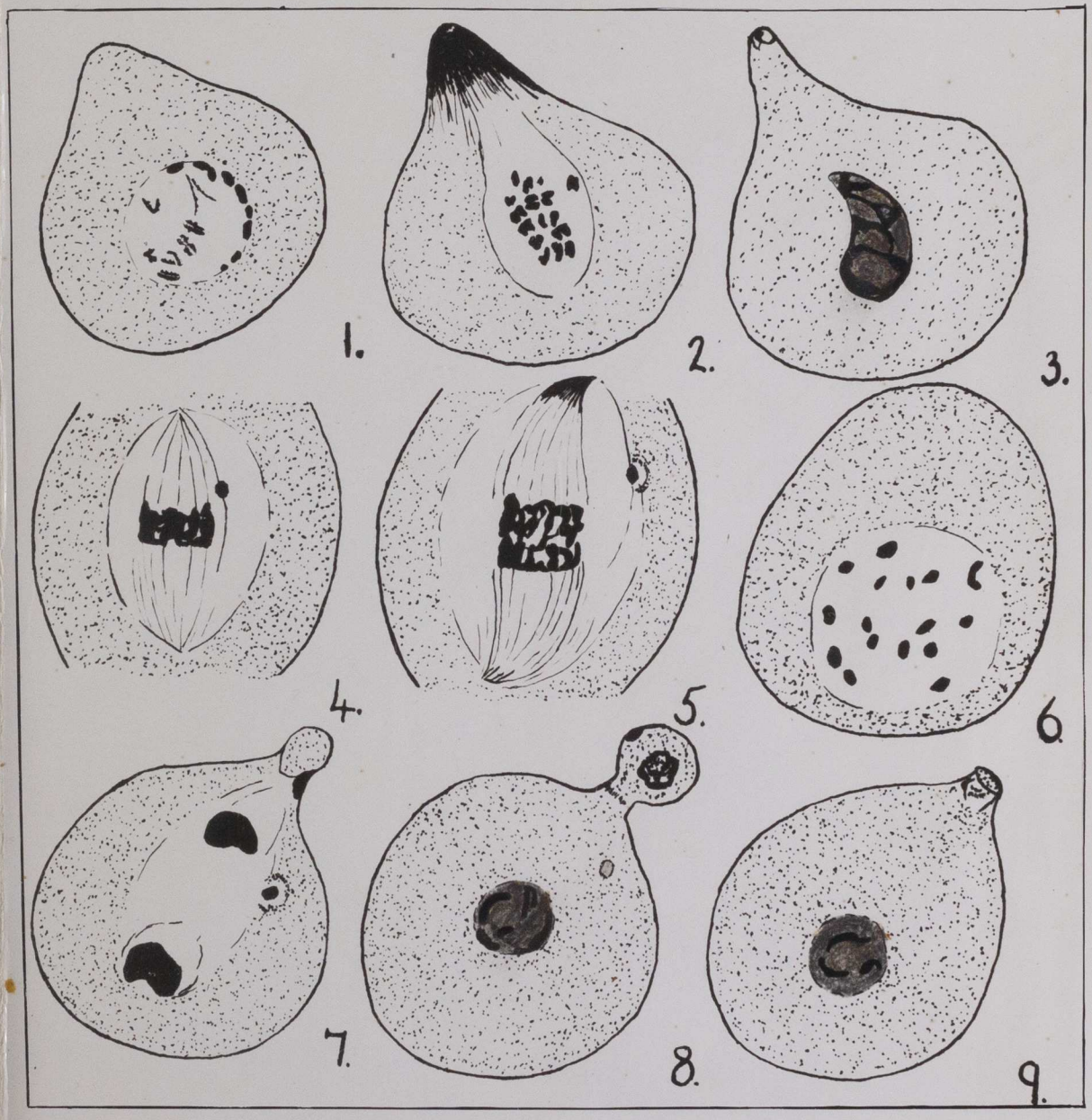
6. Metaphase, polar view; hooked-chromosome at 2 o'clock. On the original plate the latter appeared slightly out of focus at a slightly higher level. From acetic orcein preparation.

7. Late anaphase; two daughter nuclei, one about to enter bud; supernumerary nucleus still distinct; interzonal body in neck of bud; from strong acetic orcein preparation.

8. Nucleated protoplasmic bud on functional daughter cell of the spermatocyte; from acetic orcein preparation.

9. The functional spermatid showing the site of constriction of the nucleated bud; from acetic orcein preparation.

Text-fig. 1.



nuclear membrane (Plate 1, no.1) and later its metabolic character is strongly revealed by the intensity of its staining with iron haematoxylin (Plate 1, no.2; text-fig. 1, no.2). This characteristic appears to indicate considerable activity in this region probably connected with the up-piling of some of the basic structures normally associated with division. The phenomenon is also found in other bees and wasps. Moreover, some evidence that the protoplasmic elongation may contain a small centrosome near its apex is indicated in those spermatocytes stained with crystal violet. The whole process may thus be recapitulative of a normal meiosis in the abbreviated form of which the relics of a division still persist. This view gains further support from the way the chromosomes are distributed along the axis of the spindle (Plate 1, no.3), later bunching together until finally they become enclosed within a nuclear membrane. At first this restitution nucleus is somewhat elongated towards one pole (text-fig.1, no.3) but later it becomes spherical. At the end of the bunching the chromosomes have ceased to be distinctly visible and this is clearly not the result of an artefact but of the normal passage of the chromosomes into an interkinesis.

Throughout this first maturation 'division' one chromosome is slightly outstanding. The first sign of its differential character occurs at early prophase when this body, which is the largest, is seen not to have advanced in its chromatisation quite as far as the others. It is metacentric and, at this stage, may appear somewhat bow-shaped with slightly unequal arms (text-fig.1, no.1), later

becoming hook-shaped as it condenses. (Considerable variability in the shape of this chromosome occurs at this stage. Sometimes it very early assumes an attentuated hook-shape, but mostly various degrees of a bow-shape are first exhibited). The negative heteropycnosis is very noticeable on some plates. The behaviour of the chromosomes during the succeeding stage of this first maturation is not very clear on account of the anomalous nature of the metaphase. Indeed, the strongly abnormal behaviour of all the chromosomes at this stage conceals any small peculiarity this one individual chromosome may have in excess of the others. It is, therefore, only distinguishable from them by its shape and size, which become of doubtful significance once the full contraction of the chromosome has occurred. One further fact stands out clearly - the degree of nucleination of this chromosome is now not less than that of the rest of the chromosomes, so that its cycle of nucleination must have caught up with that of the latter. However, in the more advanced stages of the maturation, the chromosome again becomes recognisable, sometimes slightly understained, lying just apart from the main chromosome mass now scattered along the axis of the cell. On some occasions it appears at one end of this mass (Plate 1, nos.2 and 4), at others at its side (text-fig.1, no.2). Subsequently, however, it is always incorporated in the interphase nucleus with the other chromosomes and is thus retained for the second spermatocyte.

The interkinesis, judging from the limited number of cells

found in this state, is probably of relatively short duration. The nucleus is variable in size, and sometimes weak prochromosomes are visible within it.

Prophase of the second spermatocyte can generally be distinguished from prophase of the first spermatocyte by the omission of chromomeres and by the looser association of the dyads (Plate 1, no.5). The negative heteropycnosis of the differential chromosome makes it recognisable at this stage, and thereafter, even when it has become once more deeply staining, it stands out clearly from the rest (Plate 1, nos.6-15). Its behaviour will be considered after that of the autosomes. Following prophase the autosomes move regularly on to the metaphase plate and a 'normal' spindle is formed except that the fibres at the proximal pole are somewhat diffusely associated, whereas at the opposite pole, where they pass into a protoplasmic elongation of the cell, they are compact (text-fig.1, nos.4,5). At anaphase the components of the dyads separate, moving to their respective poles, where later they are invested with nuclear membranes. In the meantime, the elongating process, including its nucleus, rounds off to form a small but complete bud (Plate 1, no.16a) which is later constricted off as a separate non-functional spermatid. The 'neck' of the bud (Plate 1, no.16b) starts to retract once the bud has matured.

During the whole of this second maturation division the differential chromosome, as already stated, is clearly recognisable. When the autosomes move on to the metaphase plate, as well as at anaphase, it is seen to lie apart from them (text-fig.1, nos.4-6).

In preparations from material that has undergone long fixation in acetic alcohol it can be observed attached to spindle fibres, like the autosomes, but the fibres attached to the differential chromosome are 'out of true' when compared with the others (Plate 1, no.6). They exhibit a marked distinctness and also appear a little incomplete at the proximal pole but it is difficult to decide how far this last observation is accurate since the fibres attached to the autosomes are also relatively diffuse about this pole. In any case, under the conditions of long fixation the whole spindle sometimes lies in a long S-curve (text-fig.1, no.5) which tends to mask the essential features around the poles.

When the components of the autosomes (dyads) separate at anaphase the differential chromosome does not travel with them to either pole. Instead, it moves with its attached fibres to the periphery of the nuclear area (Plate 1, nos.14, 15; text-fig.1, no.5) and comes to rest there, as a dyad, at some distance from the equator and to the side of the main elongating spindle. Presumably a differential contraction of the fibres has occurred. Moreover, since the visible periphery of the nuclear area apparently coincides with the limits of the nucleoplasm, the differential chromosome is still retained within the latter. It now becomes enveloped within its own nuclear membrane forming a very small nucleus (text-fig.1, no.7) which is easily overlooked. With the dissolution of the main nuclear area it is now released into the general cytoplasm and for a time three completely formed nuclei are visible (text-fig.1, no.8). The fate of the very small nucleus with its heterochromosome is

somewhat uncertain. In a previous publication (Manning, 1949) it was described as travelling to the periphery of the cell but at that time it had been studied mainly from acetic orcein squashes. In the later observations, made on sections prepared from material that had undergone long fixation in acetic alcohol, it has never been found lying actually against the cell wall though accurate observation of this point is most difficult. Within the cytoplasm its position varies slightly, it gradually loses its staining properties and becomes no longer recognisable (text-fig.1, no.9).

No-one familiar with the material could doubt the existence of the differential 'body' described above. Even the photographs (Plate 1, nos.7-15) clearly demonstrate its presence and such figures are in no way exceptional. That it is a chromosome is evidenced by its attachment to spindle fibres. Fortunately it cannot be confused with the 'interzonal body', which is a mere relic of spermatogonial junctions on the outside of the cell (illustrated only in text-fig.1, no.7), or with a nucleolus which has quite a different nucleic acid cycle.

The result of the behaviour of this differential chromosome is that the nucleus of the functional spermatid contains only 15 chromosomes and not 16 as generally believed.

Oogenesis.

Oogonial and early oocyte configurations were studied in the ovarioles of virgin and mated queens whose ovaries had ~~previously~~

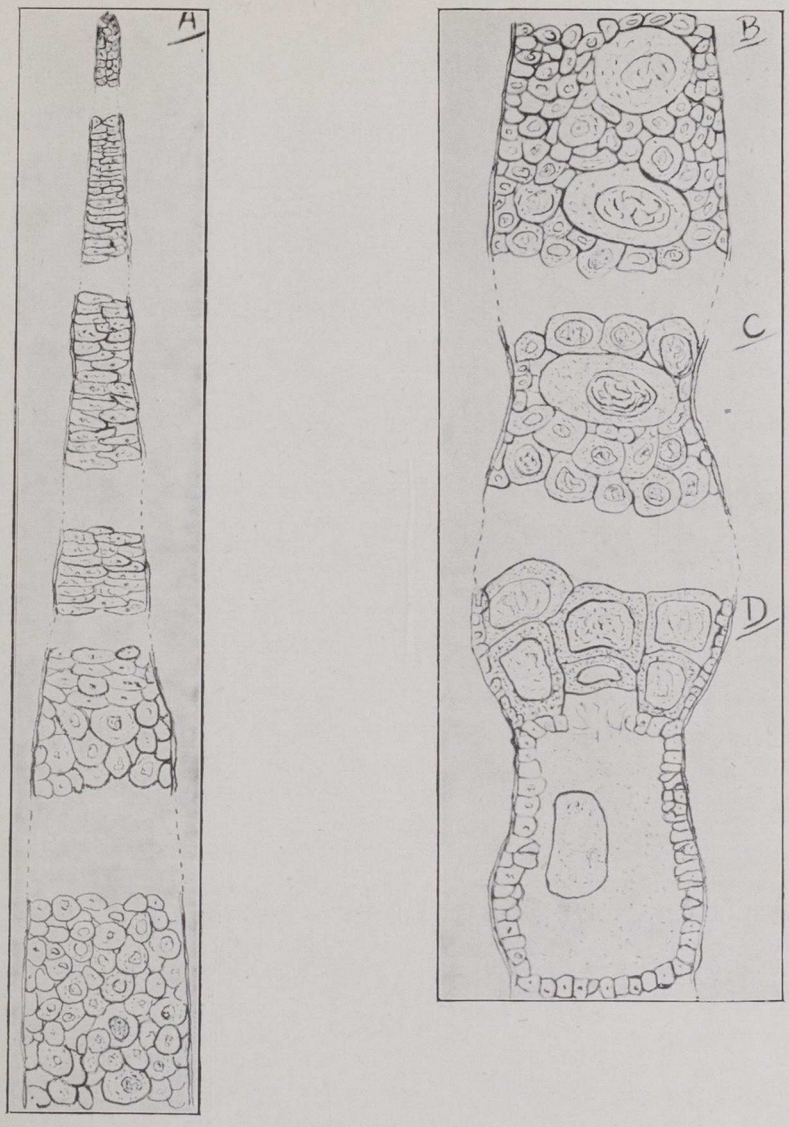
been dissected out and either made into acetic orcein smears and squashes, or fixed in the same fixatives as those used in the study of spermatogenesis.

Oocyte maturation was studied in the freshly laid eggs of queens and 'laying workers'. Those of the latter, of course, were unfertilized. Various methods of obtaining the eggs were employed, an important one being the periodic inspection of combs after destruction of the eggs they previously contained so that freshly laid eggs were not confused with older ones. In the case of the queen herself such newly laid eggs were also obtained after caging her on the comb. The most successful results, nevertheless, came from certain queens which continued to lay quite freely even after the combs on which they were laying were removed from the hive for inspection. In this case neither prior preparation nor caging was required. These queens were the very generous gift of M.A. Alber, of Sicily.

The eggs themselves were fixed on a slide by being dried in sun and air after having had their membrane pricked with a needle inorder to release the ooplasm. They were then stained in orcein (acetic) and mounted. Very good chromosome configurations were obtained by this method.

Concerning ovarioles first (text-fig. 2): in these structures it was found that all stages of egg development from oogonia to primary oocytes are continuously present. The extreme anterior region of the tubule is the zone of extensive cell proliferation (text-fig. 2,A). The oogonia derived from this region have been

TEXT-FIG. 2.



EXPLANATION OF FIGS.

A. Anterior region of ovariole showing oogonia, which later differentiate into primary oocytes, nurse cells and small follicle cells.

B. Primary oocytes, leptotene and zygotene.

C. Primary oocytes, pachytene (?), and nurse cells at similar stage.

D. Primary oocyte with "composite body." Nurse cells at stage prior to degeneration.

found to contain only 31 chromosomes (c.f. text-fig.3, no.9), one of which is large and hooked. No evidence for two such chromosomes has ever been encountered. Lower down the tubule, the gonia become a solid series of transverse cells. They soon increase in size, appearing more oval as the ovariole widens. Later, these differentiate into central cells, nurse cells and small follicle cells, the latter tending mostly to line the periphery of the tubule. The central cells become the primary oocytes (text-fig.2, B,C). The latter, during their development, increase greatly in size, far outstripping the nurse cells, and each one soon becomes invested by a follicular layer which is incomplete adjoining the nurse chamber(text-fig.2, D). At this stage the oocyte has already entered prophase, in which leptotene figures can first be distinguished, later passing into an anomalous zygotene and pachytene (text-fig.2, nos.B,C). As the chromosomes now begin to enter a diffuse or 'composite body' stage (text-fig.2,D) their interpretation presents some difficulty. They remain in this stage, moreover, until after the egg is laid. Thus the only further development which is known to take place is the increase in size of the oocyte and the simultaneous granulation of the contents of the nurse cells. These contents are later discharged into the oocyte, the cytoplasm of which is thereby tremendously increased in volume.

Once the egg is laid the germinal vesicle is readily identifiable as a large nucleus sometimes, but not always, lying in a patch of dense ooplasm (Plate 2, no.1). At this stage it much

resembles the polar nucleus of the next stage but can generally be distinguished from it by its somewhat larger size, by the complete absence of other nuclei (or configurations) in the surrounding ooplasm and by the character and disposition of the chromosomes as they once more become visible. Since the latter, however, entered the diffuse stage at about pachytene we might expect them to re-appear at this stage. In a sense they do, but what normally happens is that a dense chromatin mass, much resembling a nucleolus, obtrudes itself and, from this, chromosome arms radiate out into the nucleoplasm (text-fig.3, no.1). Other chromosomes, however, are seen at the same time to lie individually in the nucleoplasm. No trace of chiasmata has ever been witnessed in any of the chromosomes. Moreover, since only about sixteen such bodies can be traced, this observation is believed to reflect the very close approximation of homologues. The stage is thus looked upon as late pachytene or early diakinesis in which evidence for continued polarisation of close-paired homologues is apparent (Manning, 1949). This is naturally followed by the transition of the chromosomes to later diakinesis and metaphase (text-fig.3, nos.1-5) and during this time three very important phenomena are observed which call for careful examination.

In the first place there is the question of the omission of chiasmata. In an earlier paper (Manning op. cit.) it was suggested that close-pairing, such as we find in the mantid Callimantis, explained the phenomenon in Apis. This point of view is further supported here. Indeed, at early diakinesis

Explanation of text-fig. 3.

1. The 'composite body' lying in a patch of relatively dense ooplasm. The figure illustrates the chromatin knot from which chromosome arms radiate. Egg just laid.

2. The dissolution of the nuclear membrane of the 'composite body' and the re-appearance of distinct chromosomes. The thickness of the latter is due to the close pairing of homologues. The rough division of the chromosomes into two groups can be observed. X-chromosome at 9 o'clock.

3. Early diakinesis; chromosomes lying free in the ooplasm. Some denser ooplasm previously surrounding the nuclear membrane still persists. The duality of structure of some chromosomes is recognisabl X-chromosome at 6 o'clock. (c.f. Plate 2, no.2).

4. Diakinesis; the two groups of chromosomes have separated out (c.f. Plate 2, no.3).

5. Metaphase of the primary oocyte; the X-chromosome lying distinctly off plate. (c.f. Plate 2, no.4).

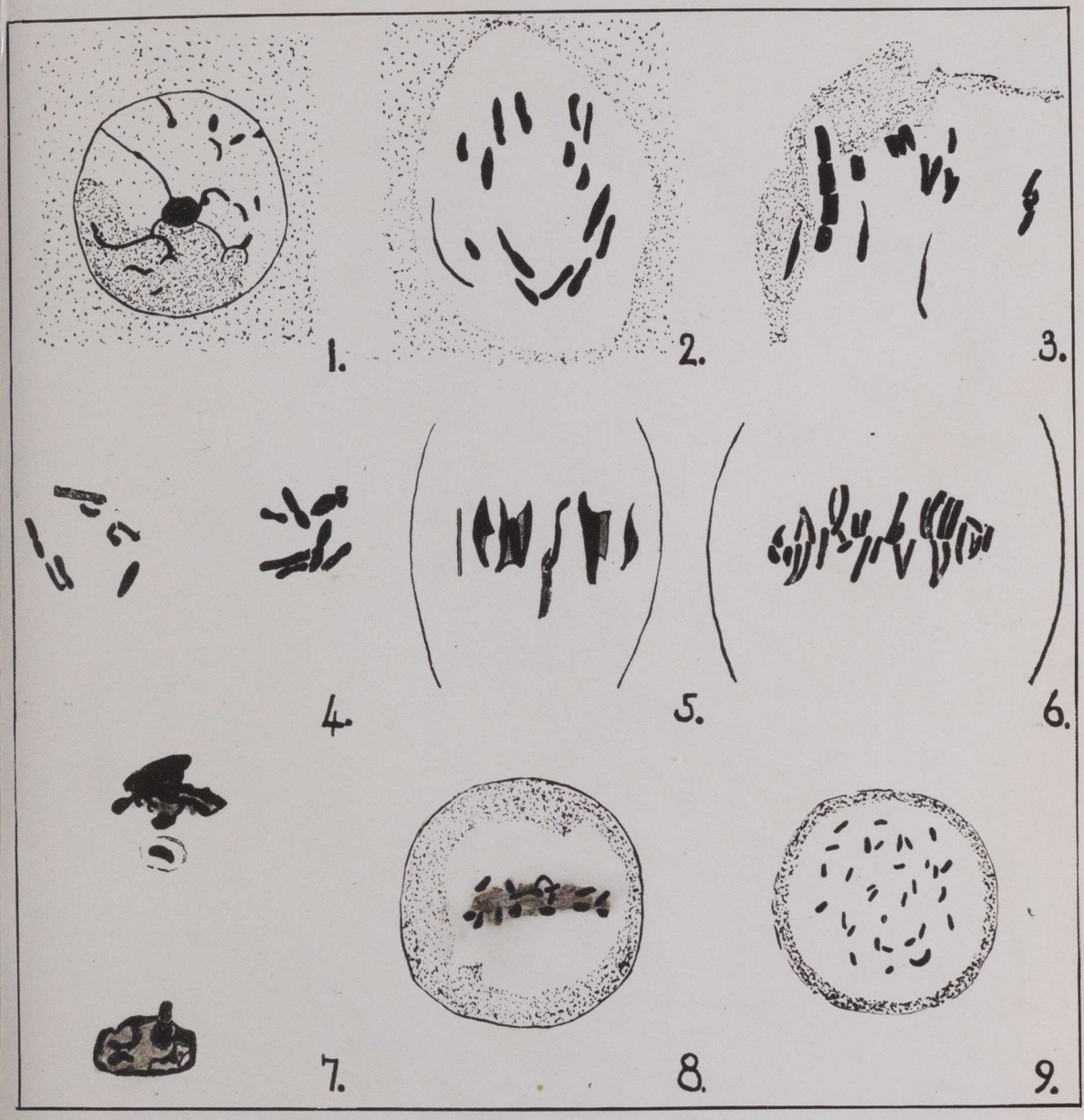
6. Early anaphase of the secondary oocyte, showing the regular separation of chromosomes. (c.f. Plate 2, no.5).

7. Early cleavage nuclei; in figure above, the X-chromosome lies off plate, whereas, in figure below, it is believed to be the one which is just entering the main nucleus mass. (c.f. Plate 2, no.6).

8. Blastoderm cell (not to scale); prometaphase side view, showing the X-chromosome, 10 o'clock, slightly delayed in reaching the plate.(c.f. Plate 2, no.7).

9. Oogonial plate (not to scale), showing 31 chromosomes; X-chromosome at 5 o'clock.

Text-fig. 3.



(text-fig.3, no.3) several chromosomes have now been found sufficiently opened out to reveal the characters of the association. There is indeed no suggestion of the presence of chiasmata or of their terminalisation, the figures being those of two intimately associating chromosomes lying near each other. This absence of chiasmata has certainly contributed to the confusion in regard to 'dyads' which permeates the literature. They have been described in both spermatocytes and in oocytes, but are, in fact, entirely unrelated structures. In spermatocytes, the 'dyad' is an association of two chromosomes still exhibiting their recent chromatid origin by reason of having only just separated enough to reveal the distinctness of the parts. In the oocyte it is an associating pair of homologues.

The second phenomenon relates to the chromosomes which emerged, still polarised (text-fig.3, no.1), from the 'composite body' stage and which now gradually come to form two groups (Plate 2, nos.2,3: text-fig.3, nos.2-4). Eventually these groups give a superficial appearance of anaphase and not prophase (Plate 2, no.3; text-fig.3, no.4). Each group consists of eight bivalents (with the one exception that the X-chromosome is not a bivalent). The assumption by Nachtsheim (1913), however, that this is anaphase has already been shown to be completely erroneous (Manning, op. cit.) since no spindle has been formed, no indiscriminate bunching of chromosomes occurs, and the 16 chromosomes are, in their general morphology, exactly like those of the haploid male set. They are

thus recognised as the diploid complement which has separated into two groups after close approximation of the homologues. The paired homologues then move quite normally on to the metaphase plate (Plate 2, no.4; text-fig.3, no.5). The existence of these two groups at this time is very exceptional and calls for some explanation. If there were one group, or two unequal groups it might well be the effect of a continued polarisation but this alone would scarcely account for two equal groups. However, at early diakinesis some of the bivalents show considerable affinity for each other (text-fig.3, no.3). This may well reflect the effects of a past polyploidy. It is, then, at least conceivable that such a residual polyploidy combined with a continued polarisation might result in the formation of two equal groups.

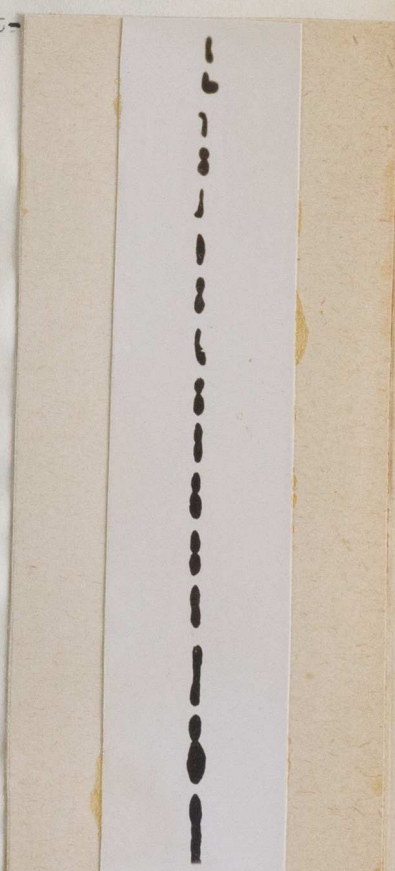
The third phenomenon to be examined is the behaviour of the X-chromosome. During the stages leading up to metaphase this can be followed quite clearly. This chromosome can first be recognised while the nuclear membrane is still intact, being more attenuated than the rest, and gradually it becomes more distinct during the progressive dissolution of the membrane (Plate 2, no.2; text-fig.3, nos.2,3). At diakinesis it is recognisable as a very slightly understained member of one of the groups. At metaphase its movement on to the plate has not quite caught up with that of the autosomes so that it arrives late (Plate 2, no.4; text-fig.3, no.5) and does not divide with the autosomes. Indeed, it does not appear at this time to divide at all in which case two types of nuclei

must result at telophase - one with an X-chromosome and one without. As the secondary oocyte nucleus, according to my observations, always forms in that half of the spindle area in which the X-chromosome lies, the secondary oocyte must contain the X-chromosome. That it does so is confirmed by evidence presented below.

The second egg maturation division is not known in the same detail as the first. This probably is due to the speed at which the chromosomes, once interphase is over, move on to the second metaphase plate. The division, however, has been ascertained as being equational in character (Plate 2, no.5; text-fig.3, no.6) and no single chromosome is outstanding on the equatorial plate. This seems to indicate that both the X-chromosome and the autosomes divide simultaneously so that their parts move in the normal manner to their respective poles. Once there, one group forms the egg pronucleus and the other group the second polar nucleus. Most careful examination has failed to reveal any exceptional features at this stage. The synchronisation of the division of the first polar nucleus with that of the second egg nucleus, mentioned by Nachtsheim, however, does not seem to be quite as exact as was once thought and the continued division of the polar nuclei, claimed by Nachtsheim, has not been confirmed. The egg pronucleus later sinks more deeply into the ooplasm, preparatory to its cleavage divisions if unfertilized, or to its fusion with the sperm pronucleus if destined to be fertilized.

Although the differential chromosome has not been recognised

at the second oocyte metaphase, its presence is often revealed at slightly later stages. First, when the nuclear membranes are forming around the new nuclei sometimes a tardiness of movement on the part of one chromosome brings about its slight separation from the rest. One rather extreme case has been illustrated on Plate 2, no.6 and in text-fig.3, no.7. Indeed, here it looks as though the delay may be sufficient to exclude it from the main nucleus. Such excessive delay, however, is not typical. ~~later~~ Second, the presence of a differential chromosome is revealed once again in early blastoderm cells when it shows a slight delay in reaching the metaphase plate (Plate 2, no.7: text-fig.3, no.8). If the account of spermatogenesis, given above, is correct this differential chromosome must have come from the female. In any case, the illustration is from a male blastoderm so that there can be no doubt whatsoever that the differential chromosome must have been present in the egg pronucleus.



Text-fig. 4.
Diakinesis bivalents
A. mellifera L..

Chromosome number and morphology.

It has already been claimed (Manning, 1949) that chromosome complements of 15A + X and 30A + X are characteristic of males and females respectively. The facts brought to light in the present studies support this view. Counts of chromosome numbers in gonia, certain

somatic cells and in oocytes and spermatocytes, as well as the unmistakable differential behaviour of the single X-chromosome, all point clearly to the correctness of the earlier conclusion.

The general morphology of the chromosomes is now known in fair detail. The idiogram of diakinesis bivalents of the primary oocyte is given in text-fig. 4 and the existence of a series of 16, ascending gradually in size, is indicated. Although the X-chromosome at other stages is distinguished by its larger size and its hooked shape, at diakinesis its contraction is such that it is no longer outstanding in size and it has become rod-shaped, but it is still sometimes recognisable by its slight understaining.

The spermatozoa.

It is generally known that during oviposition a bundle of spermatozoa is released on to the egg membrane as the egg itself passes the vaginal flap. This bundle of spermatozoa has again been observed lying on the membrane of an egg just laid. The number of individuals has been computed as not less than 10. Some, of course, have already passed across the membrane and lie in the ooplasm. Here, head and tail are at first complete, but the tail is soon lost as the advancing sperm proceeds towards the egg pronucleus. This is illustrated on Plate 2, nos.8 and 9, the former showing the sperm-path and the advancing sperm, the latter a newly formed sperm pronucleus. Supernumerary sperms quickly degenerate in the ooplasm after showing, at most, ~~but~~ slight development.

Summary.

Spermatogenesis.

1) Spermatogonial metaphase plates give a count of 16 chromosomes, as found by previous workers.

2) One large hook-shaped chromosome is shown to exhibit a differential behaviour during the maturation stages.

3) Spermatocyte maturation is anomalous but exhibits two phases: a) is the abortive phase which results in the formation of a restitution nucleus and a protoplasmic elongation, b) fulfils, as it were, the expectation of the first abortive division. It results in the production of two spermatids, only one of which is functional, the other, which is very much smaller, is sloughed off and undergoes degeneration.

The nucleus of the functional spermatid does not contain 16 chromosomes as might be expected, but 15 only, one - the X-chromosome having been extruded as a dyad during the second maturation division.

4) The primary split of the chromosomes and the separation of the chromatids have already occurred by early prophase of the first maturation phase but thereafter they continue to associate as dyads.

Oogenesis and phenomena associated with eggs.

1) Oogonial metaphase plates from cells of the anterior part of the ovariole show only 31 chromosomes.

2) One large chromosome exhibits a differential behaviour during the first maturation division.

3) Oocyte maturation, in the main, is normal in type for a diploid organism. There are, however, certain anomalies that occur during the first division: polarisation of the chromosomes is continued; there is a suggestion of residual polyploidy; and there is an absence of chiasmata between the bivalents.

The bivalents at diakinesis are 15 in number, but the full chromosome complement includes an additional univalent X-chromosome. The series of 16 is one of gradually ascending size. The univalent X is retained during the maturation for the egg pronucleus, so that the functional egg contains 16 chromosomes.

4) Sperms are released, in bundles of not less than 10 individuals, on to the egg membrane. Several sperms enter the ooplasm where one quickly rounds up into the sperm pronucleus.

Sex determination.

Sex determination in Apis mellifera L. appears to be derived from genic balance, which depends for its effects on the relationship of the X-chromosome to the autosomes. Thus the formula for maleness can be written 15A/X and for femaleness 30A/X. During spermatocyte maturation, however, the X-chromosome is eliminated so that all sperms are 15A. In the egg, on the other hand, it is retained. In consequence, all unfertilized eggs have a chromosome complement of 15A/X and thus develop into males. Fertilized eggs, however, are additionally supplied with 15 autosomes from the sperm thus making a complement of 30A/X and so develop into females. See text-fig. 5.

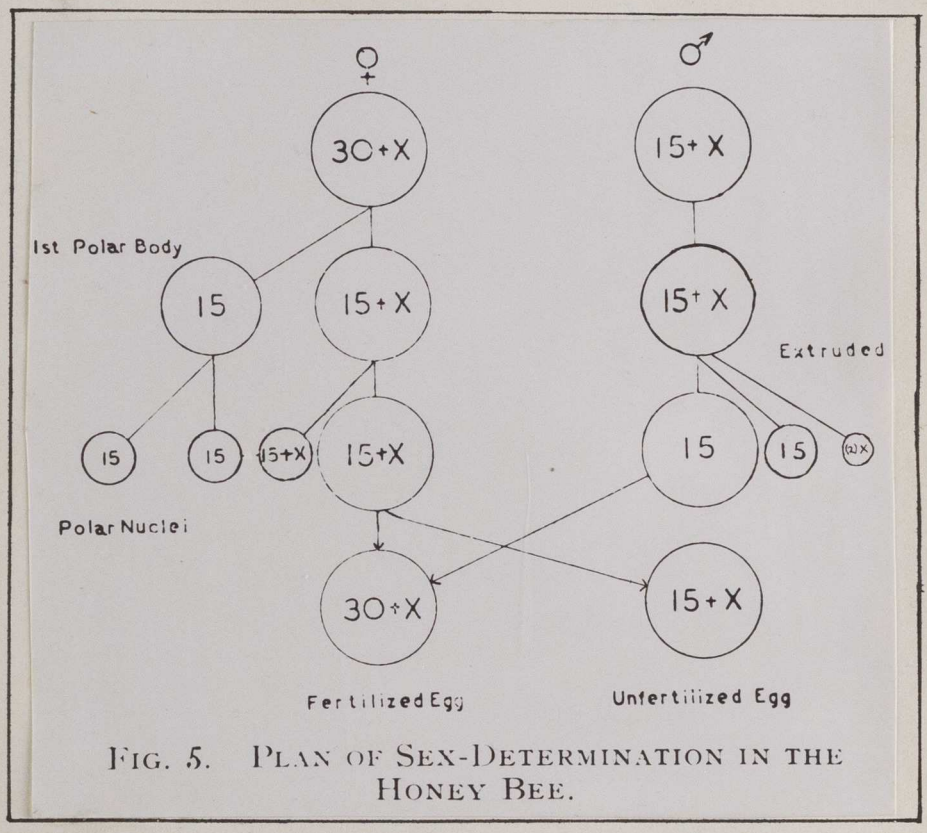


FIG. 5. PLAN OF SEX-DETERMINATION IN THE HONEY BEE.

This interpretation of the mechanism of sex determination in Apis mellifera L. brings it into line with that of other organisms and with the modern view of sex determination by genic balance. Moreover, there are no known facts - apart from Mackensen's data on viability (see p.25) - which are incompatible with the theory of sex determination here put forward. Indeed, the occasional production of females from unfertilized eggs may well be the result of a parthenogamy of the first polar nucleus with the egg nucleus - the polar nucleus containing 15 autosomes uniting with the egg pronucleus containing 15 autosomes and one X-chromosome giving the female complement of 30A/X. Moreover, obligatory thelytoky found in Neuroterus baccarum L. (see Dodds, 1939) where the female-producing eggs show some of the apparatus of a maturation division but do not produce a polar nucleus, may well have evolved from a condition similar to that of the fortuitous thelytoky sometimes found in Apis. It is quite clear, however, that the mechanism is not the same throughout the Hymenoptera since sometimes the production of such females is due to a fusion of the second polar nucleus with the female pronucleus e.g. Gilpinia (=Diprion) polytoma (Htg.) (see Smith, 1941).

(b) The Bumble Bee, Bombus terrestris (L.)

The only cytological investigation of the bumble bee, to date, is that by Meves (1903) who was chiefly concerned with the broad features of the 'Reifeteilung' or spermatocyte maturation division. He was able to establish the fact that the first 'division' is unequal, resulting in a small, anucleated cytoplasmic 'bud', while the second division, although still unequal, produces a large, nucleated 'Teilstücke'. Unfortunately, Meves' investigation was not very extensive and left many problems outstanding. Included among the latter were those of chromosome number and behaviour which the present article attempts to elucidate.

Spermatogenesis, for obvious reasons, offers the best opportunity for this study. In the present investigation, the nest of B. terrestris was removed from an earth embankment during the first week of August, its cells sorted out, and all male pupae removed. Of the latter, those in which colour was just appearing in the eyes were found to have their germ cells in the right stages of maturation. In consequence the testes were dissected out from such pupae and prepared as described for the honey bee. One of the most interesting results of this investigation has been the realisation that the cytology of the bumble bee is very similar indeed to that of the honey bee.

Spermatogenesis.

Spermatogonia are so like their counterparts in the honey bee as to warrant no special mention here. They exhibit 16

chromosomes on their metaphase plates. The last spermatogonial interphase typically exhibits two or three nucleoli though frequently these fuse into one large one.

At the end of the interphase the chromosomes rapidly take up their nucleic acid charge. Unfortunately very few good configurations of the early prophase of the first spermatocyte have been found, though what has been observed indicates the existence of chromomeres and the occurrence of an early chromatid split and separation. Thereafter, the two 'chromatids' associate together as a dyad until the metaphase of the second spermatocyte. At a slightly later stage of prophase (text-fig.6, no.1), better figures have shown more clearly the presence of these dual structures and even when they are not distinctly visible their presence is reflected in the greater width of the chromosomes themselves. One chromosome, however, has its condensation at first slightly delayed. Later this differential chromosome is often seen lying slightly apart from the main chromosome group (text-fig.6, no.2) though rejoining it at interphase. The spindle is only weakly developed and the early interphase nucleus at first assumes a 'club' shape (Plate 3, no.1; text-fig.6, no.3) prior to becoming spherical. Moreover, with the elongation of the spindle the cell itself is drawn out into an anucleated protoplasmic 'bud' or 'finger' (text-fig.6, nos.2,3). This elongation often persists well into the second spermatocyte stage indicating its very gradual retraction. There is, of course, no typical metaphase or anaphase. Centrosomes can be perceived

Explanation of text-fig. 6.

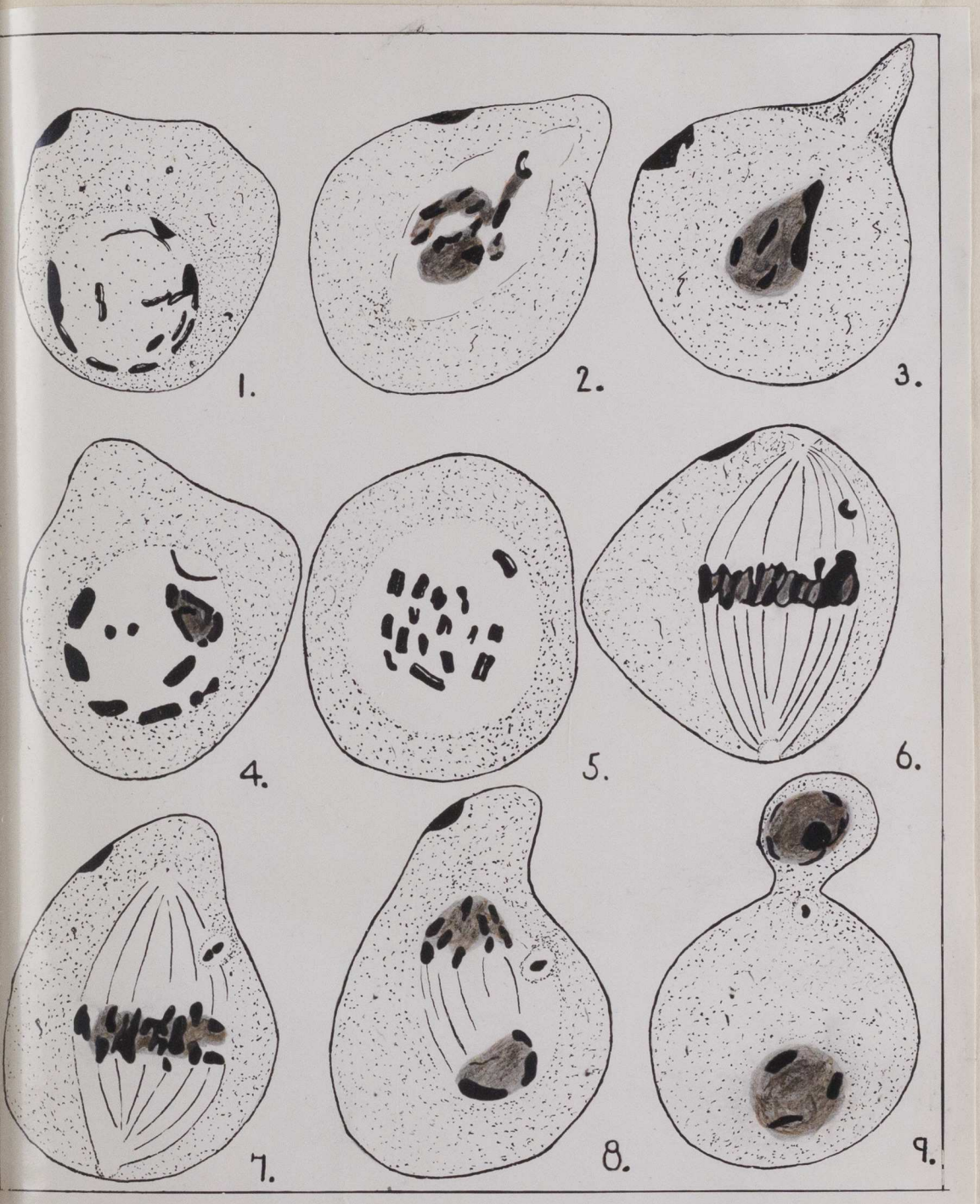
Primary spermatocytes.

1. Prophase; some dyad structures of the chromosomes illustrated; X-chromosome more faintly outlined at 12 o'clock.
2. Scatter of chromosomes along the axis of the spindle at the stage corresponding to metaphase 1; X-chromosome nearest elongating pole.
3. Early interphase, showing club-shaped nucleus and protoplasmic elongation.

Secondary spermatocytes.

4. Prophase; chromosomes not yet fully condensed.
5. Prometaphase, polar view; X-chromosome at 1 o'clock.
6. Metaphase, side view; X-chromosome attached to spindle fibre at 1 o'clock.
7. Anaphase; nuclear membrane forming round X-chromosome(s) on periphery of spindle cone, 2 o'clock; incipient development of protoplasmic bud.
8. Telophase, showing distinct small daughter nucleus and more definite protoplasmic bud.
9. Final stage of the maturation showing functional spermatid, nucleated cytoplasmic bud, and small ~~daughter~~ supernumerary nucleus near neck of bud.

Text-fig. 6



but are never large, though the one at the elongated end of the spindle appears to be the stronger. Often that at the other pole can only with difficulty be discerned. An interzonal body can be recognised as a dark patch lying on the cell membrane.

Interphase is of comparatively short duration if this is judged by the relatively rarer occurrence of cells at this stage. Prochromosomes are often in evidence.

Maturation of the second spermatocyte differs from that of the first in many particulars but principally in that it fulfils the expectation of the first by bringing about the complete separation of the components of the dyads. Moreover, there now develops a nucleated protoplasmic bud which is such a marked feature of the second maturation phase of the Apids. In the meantime, the chromosomes, having become visible again after interphase, strongly condense during their movement on to the metaphase plate (Plate 3, nos.2-4; text-fig.6, nos.4-6). The dyad structure is either continuously recognisable (see text-fig.6, no.5) or can be deduced from the excessive width of the chromosomes (text-fig.6, no.4). A normal spindle forms so that at metaphase the nuclear set-up is almost that of a mitosis, with dyads (i.e. separated but still associated chromatids) instead of normal chromatids. All autosomes reach the metaphase plate (Plate 3, no.4; text-fig.6, no.6) and at anaphase give rise to two normal groups moving to the poles (Plate 3, no.5; text-fig.6, nos.7,8). The mother cell retains one group which is destined to become the pronucleus of the sperm, while the second group proceeds into the elongating polar region, entering it

as the latter shapes itself into a bud (text-fig.6, nos, 8,9). The resulting nucleated bud, however, is apparently non-functional and is later sloughed off into the tubule and degenerates (Plate 3, nos6-8). Even in those rare cases where some further development of the bud is indicated the small spermatid presumably perishes sooner or later since small sperms are never observed among those of normal size.

While the changes described above have been taking place, during the maturation of the second spermatocyte, the differential behaviour of one chromosome (as a dyad) has been of considerable interest. After separating from the main chromosome group during pre-metaphase (Plate 3, nos.2,3; text-fig.6, nos.4,5) it rapidly completes its condensation but apparently fails to reach the metaphase plate. Instead it proceeds to the periphery of the spindle (Plate 3, no.4; text-fig.6, no.6) where it forms a small subsidiary nucleus (text-fig.6, no.7). This nucleus, in the bumble bee and in the honey bee, usually incorporates both components of the dyad. In the wasp, to be described below, two nuclei often form, one for each component of the dyad. The nucleus now either enters the protoplasmic bud, or remains in the neck of the bud, (from which it may subsequently be extruded), or it remains behind in the parent cell. All three events have been observed and no significance attaches to the fact that the one chosen for illustration shows the nucleus near or just about to pass into the neck (Plate 3, no.6; text-fig.6, no.9). In all cases the

supernumerary nucleus appears to suffer a subsequent gradual degeneration since it is rarely, if ever, observed in developing spermatids. The behaviour of the differential or X-chromosome is thus exactly the same here as in the honey bee and the end result here, as there, is that the functional spermatid contains autosomes only.

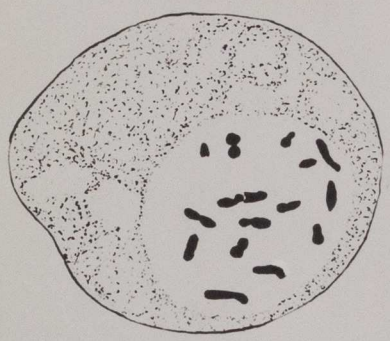
Before leaving the topic of spermatogenesis it is necessary to record several abnormalities, all rare, that have been met with during the course of the investigation. The first is the failure, in an otherwise normal spermatocyte, of a daughter nucleus to enter the protoplasmic bud, so that three nuclei come to lie in the spermatid - the two daughter nuclei and the subsidiary nucleus. The second abnormality is the attainment of a comparatively large size by a protoplasmic bud before being constricted off from the functional spermatid. Only two such cases have been seen and in both the cytoplasm of the bud was so granular that it was impossible to be certain whether or not a nucleus was present: but in the case shown in Plate 3, no.7 both daughter nuclei seem to be present within the spermatid. A third abnormality is the very small size of some spermatocytes which carry their own buds and therefore cannot themselves be confused with buds. The fourth abnormality is the occurrence of especially large cells, otherwise very reminiscent of normal spermatocytes, each with its own bud, but showing the nucleic acid diffusely scattered throughout the cell.

Oogonial figures.

Unfortunately, and for obvious reasons, it has been impossible to study oocyte figures since queen bumble bees do not lay eggs in readily accessible places and no device is known that might induce a queen to be so obliging.[x] Nevertheless, oogonial plates can fairly readily be obtained from the ovaries of queens and are most instructive. Oogonial counts give 31 as the diploid number, three chromosomes being very large (text-fig. 7A[1]). In the haploid set, however, there are two large chromosomes (text-fig. 7A[2] and B) and it would be natural to expect that four should be present in a diploid set. That three only are present is most significant particularly since two of them are a pair, the other being unpaired (see Plate 3, no.9 and text-fig. 7A[1], which both show the same metaphase plate). That the unpaired chromosome is the one which in the spermatocyte shows the differential behaviour is strongly supported by their morphology: Plate 3, no. 10 shows the spermatocyte X-chromosome isolated from the set and it is exactly the same type of large, sausage-shaped body as the odd chromosome on the oogonial plate shown in text-fig. 7A. In view of the fact that the differential chromosome is extruded from the nucleus of the spermatocyte and cannot therefore be incorporated in the zygote, it seems probable that its single X-chromosome is derived from the female as in the honey bee.

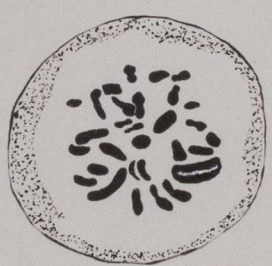
[x] Footnote. Bee World, 34 (1953) includes an abstract from Hasselrot (Agron. J. 44(4): 218-219, 1952) in which he has described such a device. It is hoped to take advantage of this during the coming year.

Text-fig. 7.



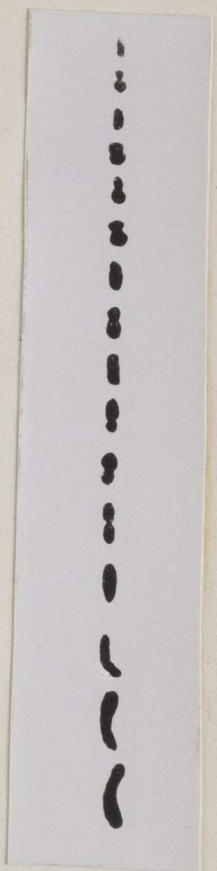
Second spermatocyte metaphase

A^2.



Oogonial metaphase

A^1.



Spermatocyte chromosomes, prometaphase, B. terrestris

Chromosome number and morphology.

The spermatocyte (haploid) number of chromosomes is 16 (text-fig. 7A[2]) and a prometaphase idiogram of them (text-fig. 7B) indicates the existence of a series of dyads, closely approximated, gradually increasing in size from a relatively small chromosome to two large ones which are approximately equal-sized. It is one of the latter which exhibits the differential behaviour and is thus assumed to be an X-chromosome. The differences shown by the idiograms (c.f. text-figs. 4 and 7B) between honey bee and bumble bee chromosomes lie mostly in variations of the relative individual sizes and in the position of their respective centromeres.

Sex determination.

The striking resemblance between the cytology of the bumble bee and that of the honey bee appears to indicate a similar mode of sex determination depending on genic balance in which the chromosome complement for the male is 15A/X and that of the female 30A/X.

Summary.

1. Bombus terrestris (L.) exhibits a spermatogenesis which, in its details, is very reminiscent of that of Apis mellifera L. and again, the differential chromosome is excluded from the nucleus of the functional spermatid. Subsidiary differences merely relate to chromosome size and individual morphology, and to the more clearly demonstrable behaviour of the supernumerary nucleus, containing the X-chromosome.

2. Oocyte maturation is not known.

3. Oogonial plates show very clearly 31 chromosomes. It has never been possible to count 32. Of the 31 chromosomes three are particularly large - two are seemingly members of a pair and the third an odd one. It seems reasonable to suppose that this odd one corresponds to the differential or X-chromosome of the male; indeed the two are strikingly similar in appearance.

4. With a male chromosome content of 15A/X and a female content of 30A/X sex determination appears to depend on genic balance exactly as in the honey bee.

3. The Cytology of some Vespoid Wasps.

Meves (1903), Mark and Copeland (1907), and Meves and Duesberg (1908) have already discussed spermatogenesis in Vespula (=Vespa) germanica (Fab.), Vespula (=Vespa) maculata (Linn.) and Vespa crabro L. respectively. These authors made careful and accurate observations as far as their techniques permitted and, though minor differences of opinion arose between them, these differences could be readily attributed either to the polemics of the age or to the difficulties of working with very small cells. The purpose of the present investigation, therefore, has been to consolidate previous observations, by the use of modern techniques, and to expand them. It has been possible to re-investigate spermatogenesis in V. germanica, and also to extend the study to V. norwegica (Fab.) and the closely related V. sylvestris (Scop.).

Nests of the various species were taken with as little disturbance as possible, either towards the end of July (V. norwegica and V. sylvestris), or early in August (V. germanica). It was fortunate that the only nest of sylvestris discovered was in a disused rabbit hutch and those of norwegica in convenient privet and hawthorn hedges, since the swift use of the chisel quickly detached the former and the clean cut of branches dislodged the latter. The nests of germanica which were frequently found in warm sandy banks offered more difficulty on account of their larger size and greater number of inhabitants. The limited use of ethyl acetate for narcotising adults diminished the danger of being stung

and apparently had no adverse affect on the pupae. All nests were removed to incubators maintained at a maximum of 25°C/ 80% relative humidity and were later studied at leisure. It was found that spermatogenesis occurs at the same pupal stage as in the honey bee and the bumble bee i.e. at the time when the eyes on the developing head begin to show slight coloration. Moreover, male pupae are readily recognised, once they are removed from their cell, by their translucent testes showing through the pupal integument and by their greater length of antennae. The testes themselves, which are relatively small, round, translucent bodies, can readily be removed from the male abdomen by pressing out the contents of the latter after a transverse cut has detached it from the thorax. The testes usually lie clearly visible in the first contents discharged. For the study of the germ cells and their inclusions, acetic orcein squashes were again found to be of great service, particularly when supplemented by sectioned material using previously mentioned cytological fixatives and stains. The germ cells themselves were very small, but nevertheless gave clear and, for the most part, adequate configurations with these techniques.

(a) Vespula germanica (Fab.)

Spermatogenesis.

Meves (1903) briefly states that in this species the first maturation 'division' results in a non-nucleated bud, and the second

Explanation of text-fig. 8.

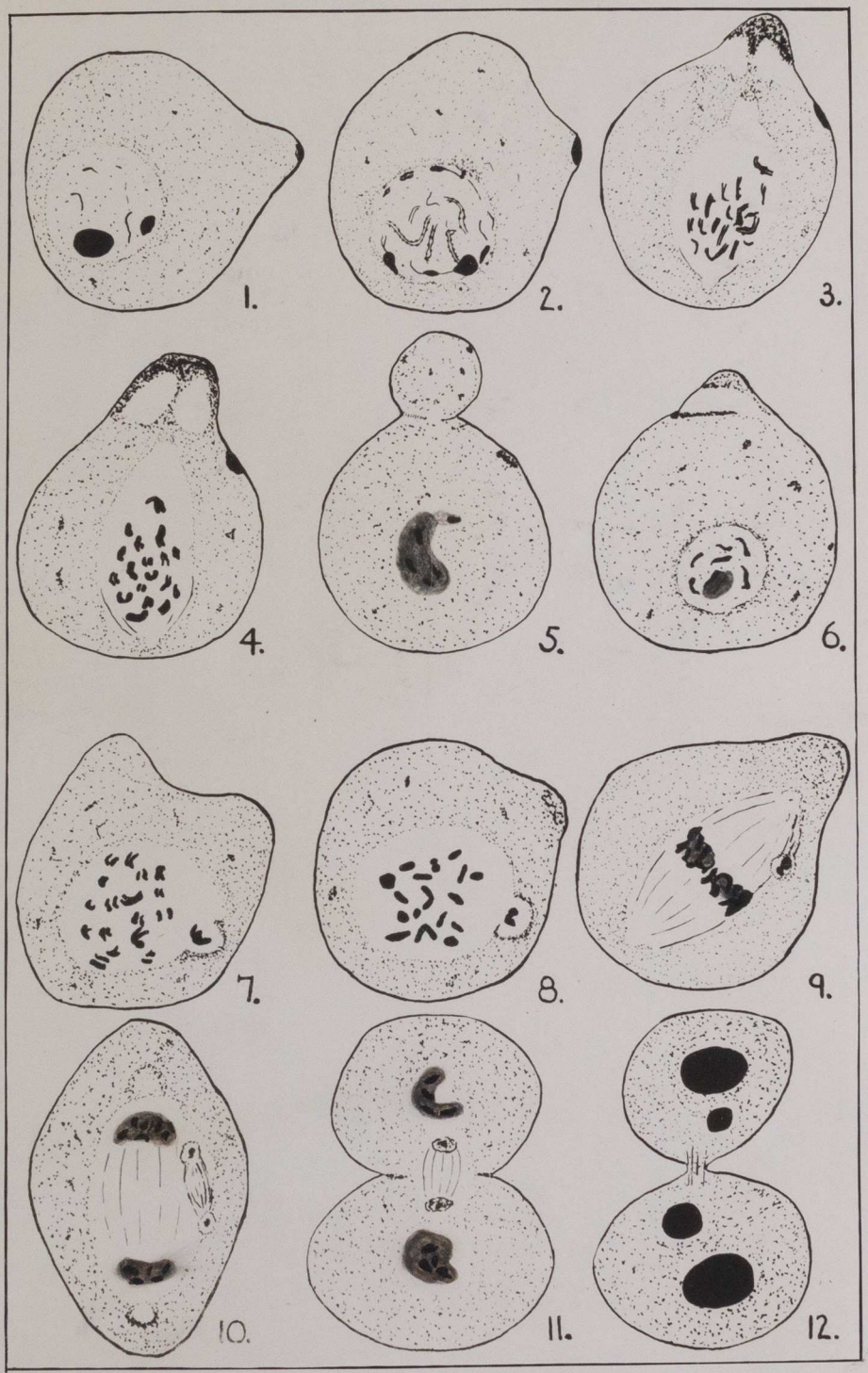
Maturation of the first spermatocyte

1. Last spermatogonial interphase; pear-shaped cell with interzonal body at apex.
2. Early prophase, showing bipartites; interzonal body at 3 o'clock.
3. and 4. Later prophase, showing condensation of the chromosomes, without a typical metaphase.
5. Early interphase; formation of an anucleated bud.
6. Late interphase; bud released, prochromosomes in evidence.

Maturation of the second spermatocyte

7. Prometaphase, oblique view; one dyad seen to be segregating from the main chromosome body.
8. Metaphase, polar view, otherwise as above.
9. Metaphase, side view, showing nuclear membrane beginning to form around the segregating body which lies at the edge of the spindle.
10. Telophase; supplementary spindle connecting two small nuclei.
11. Late telophase; daughter cells showing small supernumerary nuclei, the latter still interconnected by filaments.
12. Formation of daughter cells of the spermatocyte each containing a main and a supernumerary nucleus.

Text-fig. 8.



division in two equal-sized nucleated cells which become functional spermatozoa. These observations have been confirmed. However, the story of the maturation of the spermatocyte needs considerable enlargement since certain points were not clarified by him at this time, or even later when he and other investigators extended their studies to different species of vespoid wasps.

If we start from the last spermatogonial cell we find it well embedded in the cyst and pear-shaped, possibly as a result of the manner of its attachment to its neighbours of the same cyst. When the cell is freed after squashing, its elongated region is seen to carry the interzonal body (text-fig.8, no.1). The nucleus at this stage is large and round, and usually exhibits two nucleoli, one being larger than the other.

With the onset of maturation, the axis of the first spermatocyte is established at right angles to that of the last spermatogonial cell (text-fig.8, nos.2-5). The maturation itself is abortive in character and is accompanied by an elongation of the protoplasm which at telophase has become globular in shape (text-fig. 8, no.5, and c.f. Plate 4, no.3) and is later constricted off. Meves and Duesberg (1908) maintain that this separation does not take place in V. crabro until the succeeding anaphase. In sectioned material this has also been found to be the case in V. germanica although in squashes the bud is rarely seen to be attached after the beginning of maturation of the second spermatocyte.

The question of the centrosome in Vespula spp. and Vespa sp.

received careful attention from the earlier investigators. Meves (1903), and Meves and Duesberg (1908) believed it to be composite in structure, and thought that its movement relative to the nucleus was inhibited during first maturation. Mark and Copeland (1907), however, considered that the movement of daughter centrosomes actually took place and that it led, first, to the formation of a half-spindle, and then to a full spindle. In the present investigation evidence for poorly formed polar foci, at opposite ends of a complete but very weak spindle, has been found, and one such focal area appears to proceed into the protoplasmic elongation. This tends to support Mark and Copeland's observation. The strongly composite character of the centrosomes, mentioned by Meves and Duesberg, has not been confirmed, and the so-called 'centrioles' appear to be no more than puckerings of the cell membrane which wasp cells exhibit in fair abundance.

The chromosome behaviour is, in many ways, very reminiscent of the honey bee. At the last spermatogonial interphase, which is more or less complete, prochromosomes are only faintly visible, if at all. In early prophase of the maturation of the primary spermatocyte the chromatid split occurs (text-fig.8, nos.2-3) and it appears that the bipartites completely separate, thus becoming dyads. At this stage, too, chromomeres can often be recognised on the bipartites (text-fig.8, no.2). Since maturation of the first spermatocyte is abortive, with only a weak, ill-defined spindle, the chromosomes, as they condense, do so in an irregular

manner so that they lie scattered, in aggregate, along the main axis of the cell (text-fig.8, nos.3,4). One relatively large dyad often lies slightly apart from the rest at this stage (Plate 4, no.1; text-fig.8, nos.3,4) though it rejoins the main chromosome group at interphase (Plate 4, nos.2,3; text-fig.8, no.5). No metaphase plate is formed and the interphase nuclear membrane is at first irregular in shape (Plate 4, no.2; text-fig.8, no.5), later assuming its more globular form (text-fig.8, no.6). The interphase is probably of short duration since interphase configurations are relatively sparse. Prochromosomes are clearly in evidence (text-fig.8, no.6).

Maturation of the second spermatocyte is heralded by the rapid re-appearance of the chromosomes. At first over 30 of these can readily be counted, lying mostly in pairs (Plate 4, no.4), and undoubtedly this pairing indicates the presence of dyad structures. One large dyad segregates from the main group as the latter moves towards the metaphase plate and its behaviour is discussed more fully later. At this second maturation it does not re-join the group but proceeds towards the periphery of the nuclear area (text-fig.8, nos.7-9). Meanwhile the remaining dyads become much more closely approximated and now appear more typically as single units (text-fig.8, no.8). A normal metaphase plate and a symmetrical spindle are formed and the separation of the components of the dyads at anaphase is, in essence, that of the separation of mitotic chromatids.

During this maturation of the second spermatocyte the main spindle axis is formed roughly at right angles to that of the first spermatocyte. Moreover, the centrosomes now become relatively more easy to locate since they are associated with a normal spindle (text-fig.8, no.9). Eventually, as a result of the maturation division, two typically equal-sized daughter cells are produced (Plate 4, no.5; text-fig.8, nos.10, 11, 12). However, it is by no means unusual to perceive one cell somewhat smaller than its sister. Fortunately, this smaller cell cannot be confused with the anucleated bud, of the first maturation 'division', which is always relatively smaller still and, of course, contains no nucleus. Where the constriction occurs, which will separate the daughter cells, there is often a short waist and in this waist strong filaments are to be observed (Plate 4, no.5; text-fig.8, nos.11, 12): their significance is discussed below.

The main interest now centres on the behaviour of the differential dyad which, in wasps, exhibits several very distinctive features. First we notice that as it proceeds to the periphery of the spindle it often does so in a more distinctly 'forward' direction (text-fig.8, no.9) than is the case in honey bees and bumble bees so that here it comes to lie much nearer to one pole than it does in the bees. At this stage, too, the components of the dyad begin to move apart and at anaphase a supplementary spindle becomes visible between them. Later, nuclear membranes form round the components of the dyad. All these phenomena are illustrated

on Plate 4, no.5 and text-fig.8, nos.9-11. Most often separate membranes form round each component but sometimes, as when the dyad fails to separate sufficiently, a single nuclear membrane forms round the joint structure. In such a case only very weak spindle fibres have developed. It is quite clear that the supplementary spindle is specifically associated with the differential dyad. Indeed, the fibres themselves become most clearly visible between the two components and not on their polar sides as in the case of the chromosomes of the main spindle. It seems as though some fibres of the main spindle have been deflected, and have become re-aligned between the components of the differential dyad. However, when the main spindle has become shortened and weak, following the movement of the autosome groups to their respective poles, this supplementary spindle with its attached bodies is still strongly developed and changes its position from the side of the nuclear area to the centre, so that it now comes to lie intermediate between the two main telophase nuclei (Plate 4, no.5: text-fig.8, no.11). Here, once the supplementary spindle has arranged itself equidistantly from the poles, the new cell wall is laid down and finally cuts the supplementary spindle in two. It must not be thought, however, that the supplementary spindle at this, or at any other stage, is always of the same uniform strength. Indeed, great variation exists and when the spindle is greatly developed it is an exciting spectacle to behold.

The normal result of the processes described above is that a small, supplementary nucleus forms in each spermatid in addition

to the main nucleus (Plate 4, no.5; text-fig.8, no.12). It can later be traced there as a degenerate body. Exceptionally, when the supplementary spindle fails to take up its position equidistantly between the poles, both supernumerary nuclei are incorporated in one spermatid which thus, for the time being, contains three nuclei; at other times, when both components of the dyad are enveloped in a single nucleus, the latter which is then relatively large is distributed to one or the other daughter cells of the spermatocyte (Plate 4, no.6).

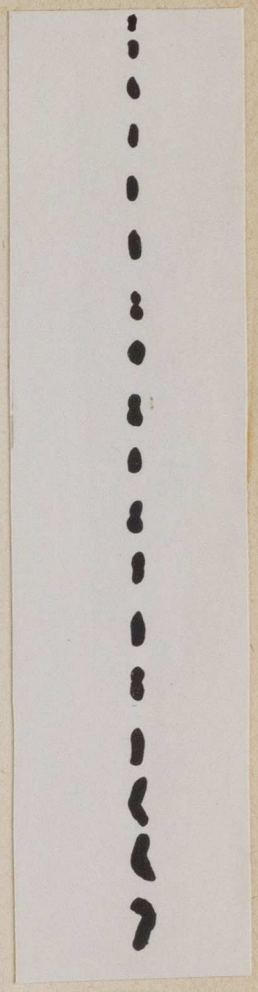
Oogenesis.

It is unfortunate that nothing is known of this aspect of the study. To obtain oocyte figures requires wasps in the process of laying, or eggs immediately they have been laid. Some fortunate chance may one day present an investigator with such material but this, so far, has not been the case. Moreover, it is not known how otherwise the material can be obtained. Stings are necessarily incurred in work of this kind but one shudders to think of the consequences that would result from opening up a wasp's nest inorder patiently to observe the queen depositing eggs, even if she obligingly proceeded with the process.

Oogonial figures can readily be obtained. They are, nevertheless, too small for critical work and no satisfactory chromosome count has been made.

Chromosome number and morphology.

It has been possible to ascertain the presence of not less than 18 chromosomes in spermatocytes of V. germanica and a prometaphase idiogram of them is figured (text-fig.9). Its most distinctive feature is the existence of three large chromosomes only one of which is differential. It must be mentioned, however, that during the maturation phases in this particular species of wasp the dyad structures are very persistent (text-fig.8, nos.2-4) and often many more than 18 distinct bodies are recognisable. Nevertheless, prometaphase and metaphase plates (polar view) have been found in which the dyads have been sufficiently closely approximated to give seemingly trustworthy counts: they have also provided the basis of the idiogram.



Text-fig.9
Spermatocyte chromosomes, prometaphase, V. germanica

Summary.

Spermatogenesis of V. germanica has been extensively investigated, using abundant material. Spermatocytes, though small, give clear and, for the most part, adequate configurations. The following important points have been noted:-

1. During spermatogenesis one chromosome shows differential behaviour very like that described for the X-chromosome of the honey and bumble bee.

2. The differential chromosome in V. germanica has a separate supplementary spindle. This strongly emphasises its differential behaviour.

3. As in the honey bee and bumble bee the differential chromosome is excluded from the main nucleus of the spermatid.

4. During spermatogenesis two functional daughter spermatids are produced, contrasting with the single functional one of the Apidae.

5. Prometaphase and metaphase plates give counts of 18 chromosomes. Three are large and similar in shape and size. One is the differential chromosome.

Oogenesis, for technical reasons, has not been investigated.

(b) Vespula norwegica (Fab.)

This species of wasp builds a smaller nest than that of V. germanica, usually in hawthorn, gooseberry or privet bushes and, in overall size, it is slightly smaller than its cousin. Fortunately, it has one most desirable asset in that, on the whole, its germ cells are somewhat larger. Since individual germ track cells, however, vary so greatly in size, the above statement is based on the relatively greater ease with which the cells were studied rather than on statistical evidence, which would demand the most careful selection of samples.

Maturation of the sperm takes place, as in other Aculeates, during the early pupal stage, when colour is just appearing in the eyes and after the cell in which the pupa lies has been capped over. To obtain male pupae at this stage it is necessary to start gathering material about the middle of July (at least in N.W.England) since the dispersal of adults from the nest occurs at the end of this month. The testes were obtained and prepared as already described in the introduction to this section.

Spermatogenesis.

Spermatogenesis, as might be expected, follows the pattern described for V. germanica. Late spermatogonial cells are pear-shaped in the cyst, and the first maturation 'division' is abortive, leading to the development of an anucleated cytoplasmic bud. The persistence of this bud until the anaphase of the second maturation division has been noted on a number of occasions; also suggestive

configurations in which the protoplasmic elongation still lingers even at telophase of the second maturation division are quite common after acetic orcein squashes. Small cytoplasmic bubbles (the 'bläschen' of Meves and Duesberg (op. cit.) have been observed but are very inconstant in position and shape, and appear to be no more than points of previous contact with adjoining cells where the cell surface has consequently become puckered. Moreover, the 'chromatoid' bodies mentioned by these authors as present in V. crabro are now recognised as the differential dyad. The behaviour of these bodies, the components of the dyad, during the maturation of the first spermatocyte shows the same sequence of events as in V. germanica, B. terrestris and A. mellifera already described. Thus they first segregate slightly from the main chromosome group but re-join it just prior to the formation of the interphase nucleus.

The division of the second spermatocyte (text-fig.10, nos. 1-12) rapidly follows on the first phase. It is equational thus leading to the formation of two, equal-sized, nucleated daughter cells. In fact the whole of the maturation process is in essence mitotic, the chromatid split having occurred not later than the early prophase of the maturation of the first spermatocyte, after which the bipartites associate together as dyads (text-fig.10, nos.1,2 and 3) until their separation at metaphase of the second spermatocyte (text-fig.10, nos.5 and 6).

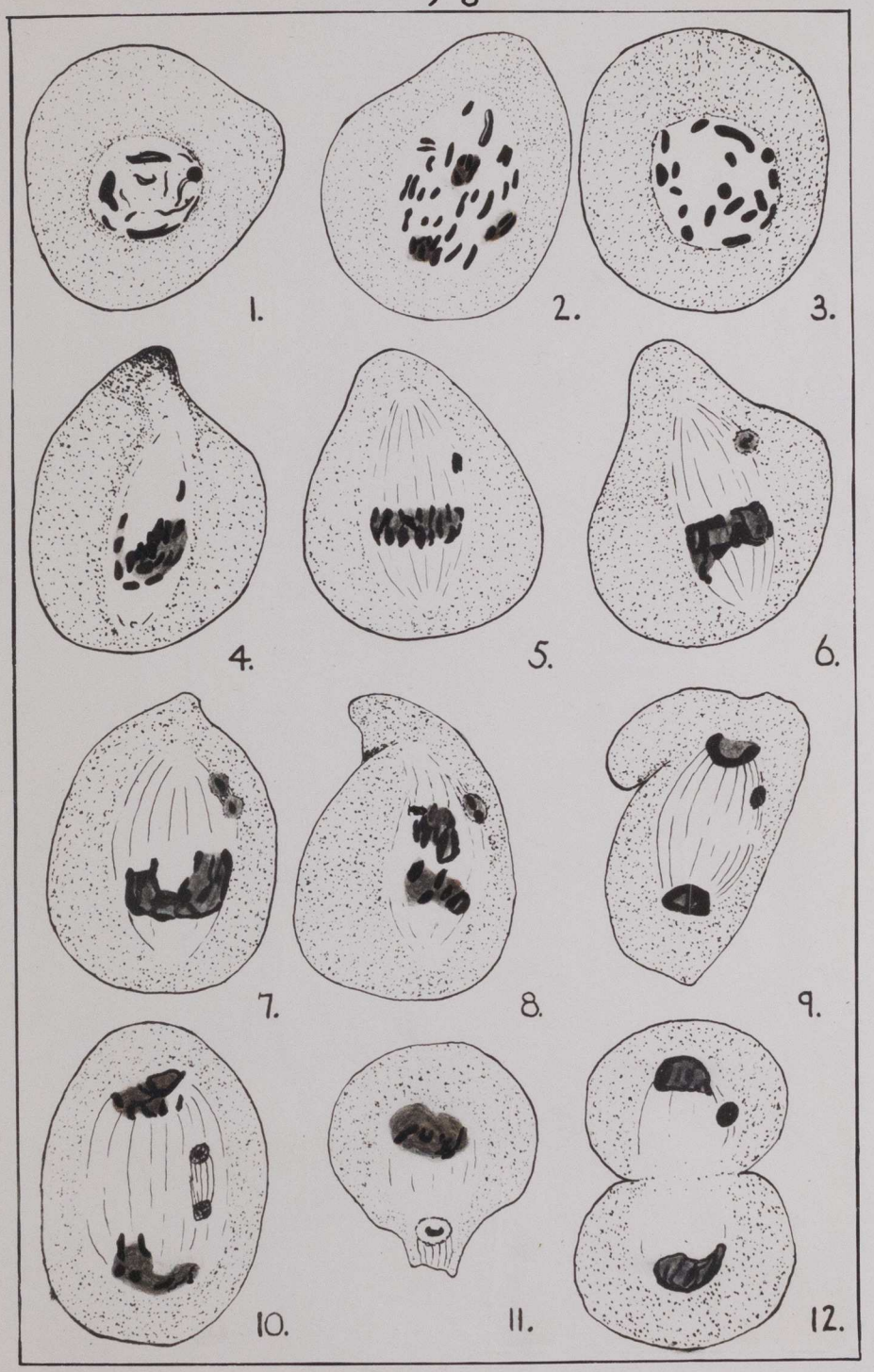
The differential dyad during the prophase of this division again segregates slightly from the other chromosomes as at first

Explanation of text-fig. 10.

Second maturation division only.

1. Early prophase; some dyad structures visible.
2. Late prophase; dyads loosely associating together.
3. Prometaphase, polar view; chromosomes rapidly condensing and losing their visible dyad structure.
4. Prometaphase, side view; a single chromosome separating from the main group.
5. Metaphase, side view, showing the symmetrical spindle, and a dyad having reached its periphery.
6. Anaphase, showing a single supernumerary nucleus forming on the periphery of the spindle.
7. Anaphase; supplementary spindle forming between the components of the dyad which lie on the periphery of the spindle.
8. Early telophase, showing cell elongating, and one main chromosome group passing the differential dyad on the periphery of the spindle. The dyad, in this case, is being incorporated within a single nuclear membrane.
9. Telophase, showing a small supplementary nucleus lying between the separating chromosome groups.
10. As above, but with the supplementary spindle connecting the two components of the dyad.
11. Spermatid, detached from sister, showing single nucleus in neck - a premature break of the sister spermatids occurred in this instance.
12. Daughter cells of the spermatocyte with a supernumerary nucleus in one of them.

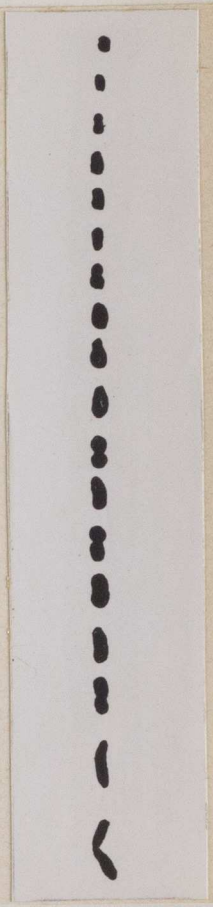
Text-fig. 10



maturation, but now it no longer re-joins the main autosome group (text-fig.10, no.4). Instead, it moves across the nuclear area (Plate 4, nos.7-10; text-fig.10, nos.4-8), coming to rest on the periphery of the spindle. There is evidence of a differential condensation of the chromosome though this is more difficult to study here, in all its phases, than in the honey bee where the cells and chromosomes are larger. The supplementary spindle discussed in the previous section on V. germanica, and so distinctly observed by Meves and Duesberg (op. cit.) in V. crabro, also occurs here in V. norwegica (text-fig.10, no.10). It is less frequently found, however, than in V. germanica and investigation reveals that in V. norwegica one supernumerary nucleus more frequently forms than two (Plate 4, no.11; text-fig.10, nos.8,9 and 12). When the spindle does occur it becomes distinctly visible between the two components of the dyad and strongly contrasts with the main spindle when the latter is becoming shorter and weaker at telophase. At this time, too, it takes up its central position between the poles so that when the cell wall is laid down between the daughter cells of the spermatocyte one supernumerary nucleus is included in each in addition to its main nucleus (text-fig.10, no.11). In the absence of a spindle, only one supernumerary nucleus is formed and it is then apparently arbitrarily distributed to one or the other daughter cell (Plate 4, no.11; text-fig.10, nos.9, 12). The supernumerary nucleus, moreover, persists for some time in the spermatid (Plate 4, no.12) before it eventually degenerates.

Chromosome number and morphology.

Finally, there is the question of the number and morphology of the chromosomes, Dyad structures once again interfere with easy counts (c.f. text-fig.10, no.2) but prometaphase and metaphase plates have been found that are suitable for this purpose (text-fig.10, no.3). Eighteen chromosomes exist. The idiogram (text-fig. 11) exhibits an important difference from V. germanica in that only one large chromosome is present - it is known to behave differentially. This contrasts with germanica where three large chromosomes exist, one of which exhibits a differential behaviour.



Text-fig.11
Spermatocyte chromosomes, prometaphase, V. norwegica

Oogenesis.

Oogenesis presents the same technical difficulties as were encountered in V.germanica. Moreover, though cells from female germ track tissue can readily be obtained, and have been studied, they are much too small for reliable assessment of chromosome number and behaviour.

Summary.

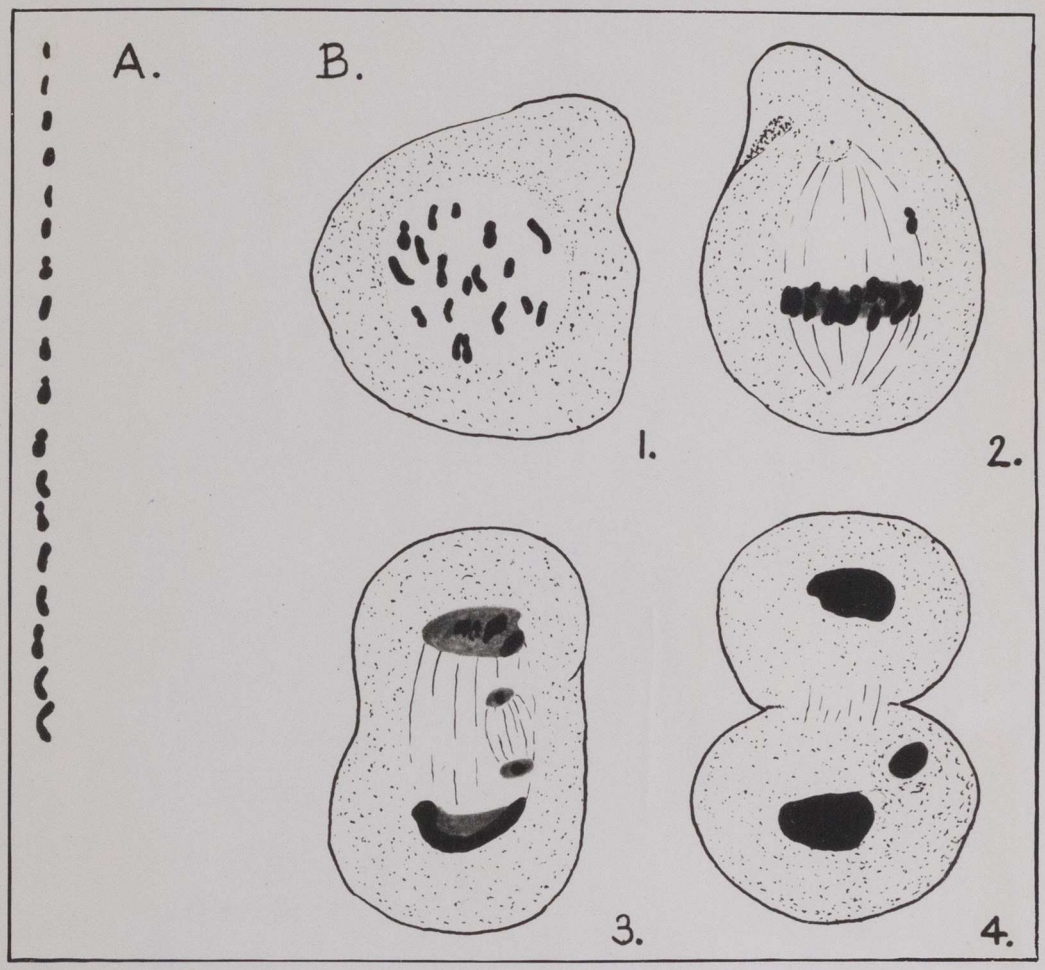
Spermatogenesis in V. norwegica has been found to be very reminiscent of that in V. germanica, and the differential chromosome here, as there, is excluded from the nuclei of the spermatids. There are two functional spermatids as in V. germanica.

Two main differences of detail in the spermatogenesis of the two species exist. The first is a more frequent occurrence of only one supernumerary nucleus containing both components of the segregated dyad in V. norwegica: the second is the presence of only one large chromosome in this species - the one which behaves differentially.

(c) Vespula sylvestris (Scop.)

Nests of this species are relatively sparse in N.W. England. It is thus very fortunate that one was discovered in a readily accessible position in a rabbit hutch. Had permission been granted to have taken the nest immediately when found, much useful material would have been obtained. As it was, the daughter of the house, through superstition, at first withheld sanction and only after much persuasion did she relax and allow the nest to be removed. In consequence of the delay, dispersal from the nest was already well advanced when it was taken so that only a few slides, containing suitable material, could be prepared. These, however, fully

Text-fig. 12



Explanation of text-fig. 12
A. Idiogram of prometaphase chromosomes, V. sylvestris.

B. Spermatocytes
1. Metaphase plate, polar view, showing 18 chromosomes.
2. Metaphase, side view; segregating dyad having reached periphery of spindle.
3. Telophase, second maturation, showing two small nuclei with a supplementary spindle connecting them.
4. Prospermatids, end of second maturation; the lower one exhibiting a small supernumerary nucleus in addition to its own main nucleus.

confirmed the expected similarity of the cytological picture to that of V. norwegica (c.f. text-figs.12A and 11, 12B and 10). And here, as in norwegica, one chromosome stands out as the largest and it is this one which exhibits the differential behaviour.

Spermatogenesis, as far as can be ascertained, repeats the processes described for V. germanica and V. norwegica. The text-figs. 12B, nos.1-4 were drawn from clear material and, among other things, give ample indication of the existence of the supplementary spindle and of the exclusion of the differential chromosome from the main nuclei.

Summary.

From the limited material available it appears that spermatogenesis corresponds closely with that of V. norwegica, no important difference having been observed.

Sex determination in vespoid wasps.

It is quite obvious that the extrusion of a differential dyad, during spermatogenesis, in these species of wasp suggests that the system of sex determination, operative here, is similar to that found in the bees. Thus 17A/X should produce males and 34A/X females. Certain of the details of the extrusion process are different between bees and wasps but this does not effect the basic mechanism of sex determination.

IV. GENERAL SUMMARY

1. Spermatogenesis has been worked out in detail in the honey bee, Apis mellifera L., the bumble bee, Bombus terrestris (L.), and the vespoid wasps, V. germanica (Fab.), V. norwegica (Fab.) and V. sylvestris (Scop.).

2. Oogenesis has been worked out in detail only in the honey bee. Oogonia have been examined in B. terrestris and in the above mentioned three species of vespoid wasps. Only in the former did their size and disposition permit of detailed study.

3. One chromosome (herein called the differential chromosome or dyad) exhibits differential behaviour during spermatogenesis in all five species studied and in all cases it is excluded from the nucleus of the functional spermatocyte.

a) In the honey bee it lags slightly behind the other chromosomes during their movement on to the metaphase plate, becomes attached to spindle fibres which lie 'out of true', migrates to the periphery of the nuclear area, and becomes enveloped within its own nuclear membrane giving a small supernumerary nucleus which is eventually lost in the cytoplasm. It is not included in the nucleus of the spermatocyte.

b) In the bumble bee its behaviour is exactly similar to that in the honey bee but the supernumerary nucleus is larger. Again it is not included in the main nucleus of the spermatocyte.

c) In all three species of wasp the differential chromosome migrates to the periphery of the nuclear area, as in

the bee, but here it normally becomes associated with a distinct and separate supplementary spindle. It is not included in the nucleus of either spermatocyte.

4. There is also a similar unpaired chromosome in the female germ cells of the honey bee and bumble bee. In wasps, with the available techniques, it has not been possible to see the details of the chromosomes in the female.

a) In the honey bee the unpaired chromosome is clearly recognisable in the oogonia and shows differential behaviour. At first maturation it lags behind the remaining chromosomes so that it arrives late on the metaphase plate. It does not divide. It is incorporated in the nucleus of the second oocyte. During the maturation of the second oocyte, however, it divides with the remaining chromosomes so that a differential chromosome is always included both in the egg pronucleus and in the 2nd polar nucleus.

b) In the bumble bee an unpaired chromosome is clearly recognisable on the oogonial metaphase plate. It has not been possible to obtain material for the study of oogenesis.

5. In the honey bee the sperm contains 15 chromosomes (all autosomes) and the egg 16 (15 autosomes and 1 X-chromosome).

The male, developed from an unfertilized egg, is 15A/X: the female, developed from a fertilized egg, is 30A/X.

Thus in the honey bee sex determination appears to be a matter of genic balance.

6. In the bumble bee, B. terrestris, the sperm contains 15 chromosomes (all autosomes). The chromosomes of the unfertilized

egg have not been observed. The male, developed from an unfertilized egg is 15A/X as in the honey bee, A. mellifera, and the female developed from a fertilized egg is 30A/X (from oogonial counts).

Thus in the bumble bee sex determination seems to be a matter of genic balance as in the honey bee.

7. In all three species of wasp, V. germanica, V. norwegica, and V. sylvestris, the sperm contains 17 chromosomes (all autosomes): the number in the egg is unknown.

The male, developed from an unfertilized egg is 17A/X. The number in the female is unknown.

The behaviour of the X during spermatogenesis is so exactly parallel with its behaviour in the honey bee and bumble bee that it seems almost certain that it is part of a similar sex determining process.

8. Additional facts of considerable cytological interest, but outside the scope of the present paper, have come to light and are recorded. They are also referred to in the Discussion.

V. DISCUSSION

The main question of interest arising from the present study is that of the determination of sex in bees and wasps. The facts that have come to light seem to make it quite clear that in the honey bee a system of genic balance is operative, just as it is in animals in general: the available evidence is extremely strong that a similar system is also operative in bumble bees and wasps. The system is dependent upon a morphologically distinguishable X-chromosome, which exhibits a differential behaviour so that it is always absent from sperms and always present in eggs.

A very similar method of sex determination is reported by Dreyfus and Breuer (1944) for the Scelionid Telenomus fariai Lima. Here a spermatogonial division, instead of continuing its normal mitosis, becomes differential in that one of the chromosomes loses a segment. This chromosome is described as the X-chromosome and the segment is believed to be the active sex segment: after its loss the remainder is termed the Y-chromosome. Thus by a regularised fragmentation and the loss of the fragment the sex potentiality of the sperm pronucleus is determined. Moreover, a differential maturation of the oocyte retains the whole of the X-chromosome for the egg pronucleus. Obviously here is a similar, but perhaps more tentative, method of arriving at the determination of sex on a system of genic balance such as is herein described for bees and wasps.

The occurrence of sex determination by genic balance in forms as widely separated as the Scelionidae on the one hand and

the Apidae and Vespidae on the other suggests that the system may be fairly widespread in the Hymenoptera.

The case of Habrobracon, of course, is different. Indeed, the system of multiple sex alleles does not fit in with any other known system of sex determination either in the animal or in the plant kingdom. Even for the Hymenoptera Whiting, who formulated the system, realised its limitations and quoted the case of its inapplicability in the inbreeding species, Melittobia chalybii Ashm.. Several authors, it is true, have tried to force their findings into a system similar to that of Habrobracon but in no case has this been shown to be entirely satisfactory.

Mackensen made claims for a similar system in the honey bee and these claims are very strong. He actually found that his sister-brother crosses gave the ratio of inviability expected on the Habrobracon system although he failed to demonstrate the presence of biparental males even in crosses specially designed for the purpose. Such males are essential to show relationship of inviability to sex. They might be completely inviable, however, and so would not reveal themselves. In their absence it is conceivable that the observed inviability is autosomal in the honey bee but, in this case, it is an extraordinary coincidence if a similar system of lethals occurs in Habrobracon and in Apis, and in the former is associated with sex but not in the latter. Mackensen's reported results stand as a challenge to the view of sex determination set forth in this paper. His view and my own seem to be mutually exclusive. The evidence, however, supporting his view is, as yet, inconclusive: clearly more

work is needed. In support of my own view there is the mass of cytological evidence recorded in the present paper, not only for the honey bee but also for the bumble bee and for vespoid wasps. It appears to make a closely knit and telling story. There is also the evidence of a somewhat similar story for Telenomus. Moreover, there are no known facts which do not fall into line with this view, apart from Mackensen's data on viability.

The mode of evolution of the morphologically distinguishable X-chromosome in the Hymenoptera is open to speculation. The general manner may reasonably be expected to have been that applicable to animals in general in which case a differential segment, on which are located both sex and secondary sex genes, would become increasingly large until its separate morphological identity finally became established. In this case, since its development in members of the Hymenoptera must have taken place within a haplo-diploid framework, it must have evolved to the accompaniment of a differential spermatogenesis and oogenesis, or the whole system would have broken down. What now of the sex alleles of Habrobracon? In view of the extremely wide distribution of the genic-balance-mode of sex determination in animals in general it is natural to look upon the Habrobracon system as an evolutionary side branch. How such a system could come to work is outside the scope of this paper to suggest: the whole system is unlike anything else found anywhere. But in so far as it depends on a multiple allelic series it is not difficult to envisage its evolution by repeated mutation of primitive sex genes which we might reasonably expect ancestral hymenopterons to have possessed.

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In the course of this investigation many subsidiary points of great cytological interest have come to light. They occur during spermatogenesis and oogenesis and appear to be of fundamental importance. They are referred to below but their full discussion lies beyond the scope of this paper.

The fact that during spermatogenesis in bees and wasps the chromosomes are present in the haploid number makes it possible to obtain a much clearer insight into the behaviour of single chromosomes during their maturation phase than is the case in species where the diploid number obtains. It has been noted that the chromatid split of the chromosomes has occurred very early in prophase, that there follows a rapid separation of the parts, and that the components of the dyads which thus result continue to associate in their pairs until the metaphase of the second spermatocyte division, showing various degrees of closeness of this association. The explanation of such behaviour lies deep in cytological theory and involves both the timing of the chromatid split and the forces of attraction holding the components of the resulting dyad together.

Phenomena appearing during the extrusion of the differential dyad from the nucleus of the spermatocyte also call for special notice. In the wasps there is a well developed supplementary spindle associated with this dyad but in the bees there are only some displaced spindle fibres. The interest of these phenomena relates both to their participation in the process of the extrusion of the differential dyad and also to their own intrinsic nature, origin and mode of evolution.

The cytoplasmic buds, so well known during spermatogenesis in members of the Hymenoptera, present some fascinating problems in connection with bees and wasps. The globular character of the cytoplasmic projection during the first maturation 'division' in wasps and its ultimate separation from the parent cell suggests a less complete departure from a normal division than does the mere finger-like process found in bees, which is simply re-absorbed into the main cytoplasm. Also, during the maturation of the second spermatocyte, the development of two functional spermatids in wasps is clearly less removed from 'normal' than is the condition in the bees where one daughter cell, although possessing a complete nucleus, is simply a small, non-functional bud. The occurrence of these small nucleated buds in the Apidae certainly calls for some explanation - one which takes into account intrinsic factors which may be peculiarly applicable to bees. A suspicion arises that the reduction of one 'spermatid' to a mere bud, and the reduction of the supplementary spindle of the wasps to a mere non-alignment of certain fibres of the main spindle in the bees, may not be unrelated phenomena. In innumerable ways the spermatogenesis of wasps seems to be less modified than that of bees and if the supplementary spindle of the wasps is the precursor of the non-aligned fibres of the honey bees than its reduction and the reduction of the main spindle, with the associated unequal division, may be part of one and the same phenomenon. Why such a condition with its drastic reduction in the number of spermatozoa has survived is another problem.

During oogenesis (known only for the honey bee) one of the most interesting phenomena is the failure of chiasmata to form. The homologues pair and closely associate but no cross-over takes place. In view of the fact that no pairing, and therefore no chiasmata, can occur in the haploid spermatocyte, there must be a serious curtailment of variability in the honey bee. In social insects this limitation may be of some importance in the canalisation of those factors contributing to the development of the colony.

The continued polarisation of the chromosomes during oogenesis is also a point not without interest. Its cause is unknown. Its effects, of course, are to some extent masked through the interposition of the 'composite body' stage but it may be responsible for the orientation of the differential chromosome so that this chromosome lies on that side of the metaphase plate which permits its incorporation only in the primary egg pronucleus.

Lastly, in connection with the above polarisation of the chromosomes there are the complicating effects of what seems to be a residual polyploidy. Thus, during oogenesis in the honey bee, the 16 bivalents (the X-chromosome counting for the present as a bivalent though really a single chromosome) separate into two sets of 8 bivalents each. One possible explanation for such behaviour is that it may reflect the effects of a past polyploidy. It is thus interesting that in the stingless bees, the nearest cousins of the honey bee, the chromosome number is 9 (Kerr, 1948).

Any one of the above subsidiary points could lead into much wider fields of study but they have no obvious and direct bearing

on the sex-determining mechanism of bees and wasps, which is the theme of the present thesis.

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EXPLANATION OF PLATES

Plate 1

Spermatocytes of honey bee.

Maturation of the primary spermatocyte.

1. Prophase, showing protoplasmic elongation of cell and also the commencement of the elongation of the nuclear membrane at one pole; circa X 3000.
2. Metaphase (abortive), showing the scatter of chromosomes along axis of spindle and distinct polar cap; iron haematoxylin; circa X 3000.
3. Metaphase (abortive), showing the scatter of more distinct chromosomes; 'hook-shaped' chromosome at top of plate; circa X 4500.
4. Metaphase (abortive); chromosomes beginning to bunch; circa X 4500.

Maturation of secondary spermatocyte.

5. Prometaphase, polar view; the association of the dyads still recognisable; X-chromosome somewhat out of focus, 6 o'clock; circa X 6000.
6. Metaphase, showing X-chromosome attached to its spindle fibres, the lower one of which is incomplete; iron haematoxylin; circa X 3000.
7, 8, 9, 10, 11, 12, 13. Various views of prometaphase, metaphase and early anaphase, indicating the position of the X-chromosome; circa X 4500.
14. The nuclear area, anaphase, with the X-chromosome having almost reached its periphery, 5 o'clock; circa X 4500.
15. Anaphase; X-chromosome on periphery of nuclear area, 8 o'clock; circa X 4500.
16a. The functional cell of the spermatocyte with small, non-functional bud attached; circa X 3000.
16b. The bud revealing the 'neck' by which it is attached to the functional spermatid; circa X 4000.

(All preparations stained acetic orcein except where otherwise stated

Plate 2

Figures from eggs of the honey bee, showing mainly egg maturation.

1. Germinal vesicle, egg just laid; circa X 1500.
2. Early diakinesis; X-chromosome 6 o'clock (c.f. text-fig.3, no.3).

3. Diakinesis; two groups of 8 chromosomes each clearly visible.
4. First metaphase; the X-chromosome lying slightly off the plate. One pole is seen to be rapidly expanding.
5. Early second anaphase, showing the quite normal separation of chromosomes from the metaphase plate.
6. Early cleavage nucleus, showing the X-chromosome lying somewhat detached from the main group.
7. Blastoderm cell, prometaphase side view, showing the slight detachment of the X-chromosome, 10 o'clock.
8. Sperm and sperm furrow in ooplasm.
9. Sperm pronucleus, remnants of tail still visible.

(All figures, except 1, circa X 3000.)

Plate 3

Figures from the germ track of the bumble bee, Bombus terrestris, showing secondary spermatocyte maturation except where otherwise stated.

1. Early interphase nucleus, 'club-shaped'; circa X 4000.
2. Prometaphase, polar view; X-chromosome detached at 2 o'clock; circa X 4000.
3. Prometaphase; X-chromosome at 12 o'clock; some dyad structures still recognisable; circa X 4000.
4. Metaphase, side view; X-chromosome at 1 o'clock, its dyad structure distinct; circa X 4000.
5. Telophase; nuclear membrane forming round X-chromosome; circa X 4000.
6. The daughter cell of the spermatocyte with protoplasmic bud attached and a small supernumerary nucleus near the neck of the latter, circa X 3000.
7. An extraordinarily large, protoplasmic bud, almost detached from sister cell; circa X 3000.
8. Nucleated bud completely detached; circa X 4000.
9. Oogonial chromosome complement, containing two large autosomes (A) and a single X-chromosome (X) (see text-fig. 7A[1]); circa X 5000.
10. X-chromosome isolated from its spermatocyte complement; circa X 7500.

Plate 4

(Fab.)
Vespula germanica, spermatocyte maturation.

1. Metaphase of the abortive first maturation phase, showing the scatter of chromosomes prior to the formation of the nuclear membrane; circa X 7500.

2. Early interphase nucleus exhibiting its typical 'club' shape; circa X 7500.

3. Interphase; protoplasmic bud formed and one large chromosome still detached from the main group; circa X 4000.

4. Late prophase chromosomes; persisting dyad structures give rise to false counts unless great care is taken; circa X 8000.

5. Two daughter cells of the second spermatocyte, showing large and small nuclei in each cell, and a supplementary spindle linking the latter; circa X 3500.

6. Daughter cell of the second spermatocyte showing two nuclei. The supernumerary one, from its relatively large size suggests that it has incorporated both components of the segregated dyad; circa X 3500.

Vespula norwegica (Fab.), spermatocyte maturation.

7, 8, 9, 10. Prometaphase and metaphase of the second spermatocyte, showing the segregation of a chromosome (dyad); circa X 7500.

11. Telophase of the second spermatocyte, showing not only the two normal telophase nuclei but in addition a single, smaller supernumerary nucleus; circa X 4000.

12. Spermatid, clearly showing a supernumerary nucleus; circa X 3000.

Plate. 1.


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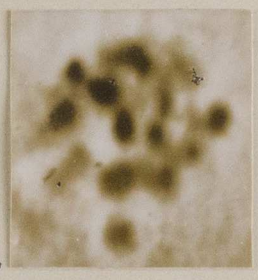
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Plate 2.


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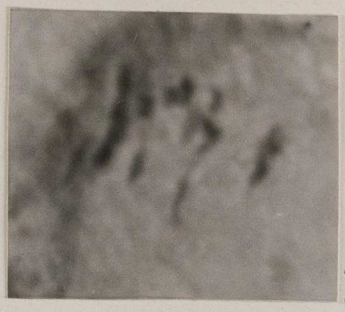
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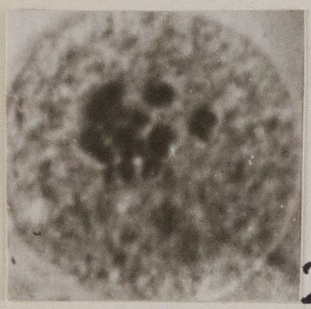
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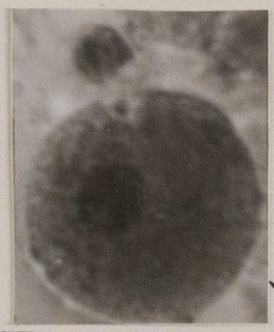
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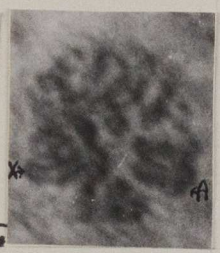
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Plate 4.


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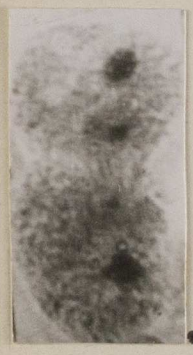
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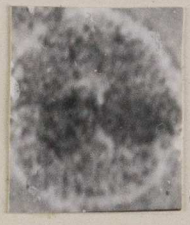
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